ABSTRACT

WILLIAM C. GREEN. A Mass Spectrometry/Mass Spectrometry Method for the Analysis of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans. (Under the direction of Dr. M. J. Charles)

Mass spectrometry/mass spectrometry (MS/MS) has been shown to be useful in the analysis of environmenatl samples for low (ppt) levels of compounds like polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in cases where interfering materials present in the samples made analysis by high resolution mass spectrometry (HRMS) difficult or impossible. While the technique shows promise because of its high selectivity, researchers have reported stability problems with mass spectrometry/mass spectrometry instruments and other questions regarding the reproducibility of analytical results exist. This study was undertaken to develop an MS/MS method for the analysis of PCDD/Fs, determine the stability of an MS/MS instrument over a period of months and to compare analytical results obtained by MS/MS with those obtained by HRMS. The results demonstrated that by following the protocol developed in the study, an MS/MS instrument can generate reproducible analytical results over a period of months and that quantitative results obtained by MS/MS were not significantly different from those obtained by HRMS.

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CID	collision induced dissociation
HRGC-MS	high resolution gas chromatography-mass spectrometer
MRM	multiple reaction monitoring
PCBs	polychlorinated benzenes
PCDD/Fs	polychlorinated dibenzodioxins/dibenzofurans
SIR/M	selected ion recording/monitoring

Introduction to Mass Spectrometry/Mass Spectrometry

Mass spectrometry/mass spectrometry (MS/MS) has become a widely used research tool in the last two decades. Applications of MS/MS include the elucidation of the structure of molecules, the exploration of ion-molecule interactions in the gas phase, and investigation of increased selectivity for targeted component analysis (McLafferty, 1983).

One type of MS/MS experiment, on a beam instrument, is performed by selecting the parent ion in the first stage of analysis, and then analyzing the product ion(s) in the second stage of analysis (Busch, 1988). Each of the mass analyzers act as sequential and independent gates through which ions must pass to be detected. The value of the MS/MS experiment is that it allows for the performance of highly selective analyses. Selectivity in the MS/MS experiment is achieved by properly setting the first gate to define the ions emerging from the source and the second to determine the masses of secondary ions generated in the reaction region between the two analyzers (Cooks, 1983). Progeny ions form as a result of the dissociation of the parent ions. This dissociation is enhanced when the parent ions enter a reaction region to collide with a neutral target gas in a process called collision induced dissociation (CID).

The ability to enhance selectivity has generated significant research interest in MS/MS for environmental analyses because extracts of environmental samples can be

made up of complex mixtures of compounds that present analytical problems when using low resolution and high resolution mass spectrometry (HRMS) (Busch, 1988).

The use of MS/MS as a rapid screening technique for identification of 114 EPA priority pollutants (phenols, aromatic hydrocarbons, phthalates, and chlorocarbons) was thoroughly investigated by Hunt et. al.(1983). These investigators demonstrated the power of MS/MS to perform direct sample analysis without extensive sample preparation or the use of gas chromatography for separation. Complex mixtures ranging from coal liquids to biological matrices have been successfully analyzed by the use of a direct probe into an MS/MS instrument (Cooks, 1983). Direct probe-MS/MS as a means to determine isomer specificity was explored prior to the development of high resolution gas chromatography (HRGC) methods. Direct probe analysis has the limitation that isomer information is not easily and directly obtainable by this method as was hoped because many isomers of compounds have identical fragmentation patterns. Since its development, high resolution gas chromatography (HRGC) has made it possible to identify more isomers and thus HRGC/MS is commonly used to analyze volatile compounds. The oil industry uses GC/MS/MS to screen drilling samples for geochemical biomarkers (steranes and triterpanes) in situations where biomarkers are present in extremely low concentrations or interferences from other hydrocarbons preclude the use of GC/MS (Isaksen, 1992). The MS/MS method is also used to identify environmental pollutants ranging from those found in paper mill effluents to those listed in indoor air pollution studies (Busch, 1988), and to analyze polyhalogenated dibenzo-*p*-dioxins and

dibenzofurans (PCDD/Fs) (Harvan, 1981; Reiner, 1989, Slayback 1983; Tondeur, 1987; Charles, 1990).

The presence and fate of polychlorinated dibenzo-*p*-dioxin and dibenzofurans (PCDD/Fs) in the environment is an important topic because of the health hazards posed by these compounds for humans and other species. Short-term health effects on humans exposed to relatively high levels of PCDD/Fs include chloracne, liver enlargement, headaches, nausea, and damage to nerve fibers (Paddock, 1989). Long term health effects from PCDD/Fs exposure are still not completely understood, but the following have been determined by researchers and documented (Paddock, 1989): 2,3,7,8-TCDD and other PCDD/Fs are cancer promoters rather than initiators. Birth defects from exposure to PCDD/Fs have been documented in cases where the mother was exposed to high doses while pregnant. The mutagenicity of 2,3,7,8-TCDD is very weak and most researchers have concluded that it is not a mutagen. While demonstrating high toxicity and carcinogenicity in research animals, PCDD/Fs (particularly 2,3,7,8-TCDD) have recently been shown to have more subtle effects (Schmidt, 1992). Increasing evidence suggests that these compounds' ability to disrupt normal immune system function rather than carcinogenicity may represent their greatest threat to public health (Schmidt, 1992). While the story on health effects of PCDD/Fs is still unfolding, the current toxicological information and the widespread presence of these materials in the environment in very low concentrations has created the need for sensitive, selective, and accurate mass spectrometry methods for quantification of these materials in environmental samples. Using MS/MS as a

quantitative tool and the analysis of PCDD/Fs is a focus of this research.

Mass Spectrometric Analysis of PCDD/Fs

Mass spectrometric analysis of polychlorinated-dibenzo-*p*-dioxins/furans (PCDD/Fs) has undergone many refinements over the past fifteen years (Huang, 1991). High resolution mass spectrometry (HRMS; resolving power 10,000 or greater) has become the method of choice for PCDD/F analysis for most laboratories because of its accuracy and selectivity compared to low resolution mass spectrometric methods (Harvan, 1981). Analysis of PCDD/Fs by HRMS is conducted using selected ion monitoring or recording (SIM or SIR). Rather than scanning the full mass spectrum of the analyte of interest, in this mode only one or two of the most intense ion masses are monitored, resulting in a 10^3 increase in sensitivity over full scan. The ability to sustain precise mass selection in high resolution SIR is made possible by monitoring an ion from the mass spectra of a reference compound (e.g. lock mass) that is continually introduced into the ion source of the instrument throughout the analysis. For PCDD/F analysis, perflourokerosene (PFK) is used as the reference compound. A different PFK ion mass is monitored for each PCDD/F congener group, and the mass chromatogram of the PFK ion mass is used to check the stability of the instrument during SIR analysis. High resolution mass spectrometry analysis of environmental samples for PCDD/Fs does, however, have limitations.

In the environment, PCDD/Fs typically are encountered at concentration levels (parts per trillion or quadrillion) much lower than other types of environmental pollutants (as much as six orders of magnitude). Polychlorinated-dibenzo-*p*-dioxins

and dibenzofurans generally are incidental by-products of incineration or chemical reactions and processes used in the production of paper, wood, pesticides, and PCBs. The presence of other materials in the sample can affect the mass spectrometric analysis in two ways. One possibility is that a compound can act as a mass interferant (i.e., the compound has the potential to interfere with the analysis of another when the difference between the masses of the ions cannot separated at a given resolving power ($m/\Delta m$). At a resolving power of 10,000 many common mass interferants (e.g. hexa, hepta, octa, nona PCBs and tetra chlorinated methoxybiphenyls) are eliminated (Tondeur, 1987). Elimination of some mass interferants can, however, require resolving power of 40,000 or higher (e.g., the molecular ion of hexa-PCB interferes with the $(M+2)^+$ ion of PeCDD and requires a resolving power of 49,000). While current instrumentation is capable of higher resolution (50,000-100,00), the subsequent decrease in sensitivity at these resolving powers makes such analysis impractical. The identity of PCDD/Fs is confirmed by comparing the measured ratios between two ions in a chlorine isotope ratio and the theoretical value. The presence of PCDD/Fs is confirmed when these ratios agree within $\pm$ 15%. It is assumed that PCDD/Fs are absent if the values exceed these limits. It is possible, however, that this deviation is caused by the presence of mass interferants.

The second possibility is that the compounds that are extracted during sample preparation and co-elute with PCDD/Fs can be present in sufficient quantity to cause instability in the source (or source de-tuning) (Tondeur, 1984). The detuning

phenomenon, also called a matrix effect, is related to the type of sample from which PCDD/Fs are extracted. The materials causing the problems are not always identifiable, but it has been shown that source instability will arise when significant amounts (50 ng/s) of such material reach the ion source (Tondeur, 1984). The result will be a deflection in the mass chromatogram of the PFK ion and a problem with sample quantitation if the matrix effect materials coelute with analytes.

Chromatographic cleanup techniques developed to remove these interfering effects are not 100% effective (Tondeur, 1987). Thus, some environmental samples may be difficult to analyze by HRMS. Source detuning as described earlier can also cause unacceptable drift in the lock mass invalidate the signal response observed in the channels monitored. To permit accurate quantitation, removal of interferences is a necessity and may be achieved by improving the cleanup procedure or improving the selectivity of the instrumental analysis. The source detuning caused by matrix effects can only be resolved by an improved sample cleanup procedure. Removal of mass interferences from a sample is possible by increasing the selectivity of analysis. The promise that mass spectrometry/mass spectrometry has shown in achieving this last objective led researchers to investigate its use the analysis of PCDD/Fs (Huang, 1991). Mass spectrometry/mass spectrometry offers greater analytical selectivity by providing the opportunity to monitor an ion that is a constant neutral loss and that is unique to the analyte. The detection limit of such a method is affected by parameters that have an effect on the dissociation of the parent ion. The screening ability of MS/MS has allowed accurate analysis of PCDD/Fs in the presence of a 1000-fold

excess of mass interferant PCBs (Tondeur, 1987.)

Parameters that Affect Collision Induced Dissociations

Performing MS/MS analysis on PCDD/Fs involves transmitting the most intense ions in the chlorine isotope cluster of the parent ion in the first stage of analysis, inducing dissociation collisions of the parent ion with a target gas, and then detecting specific product ion(s) in the second stage of analysis. In this study, the product ions monitored were those formed by the loss of $CO^{35}Cl$ from the parent.

In the CID process, translational energy of the parent ion is converted into internal energy by collision with the neutral target gas. Additional translational (kinetic) energy available for conversion into internal energy upon collisions is imparted to the parent ion by exposing it to a potential difference within the collision cell (Busch, 1988). This potential difference is called the collision energy. The collision energy, designated E_{lab}, represents the total amount of translational energy the parent ion has in the reaction region. The actual amount of translational energy available for conversion into internal energy, however, is determined by the mass of the parent ion (m_p) and the mass of the target gas molecule (m_g) involved in the collision. The center-of-mass collision energy (E_{com}) equation is used for this determination:

$$E_{com} = E_{lab} * \frac{m_g}{m_g + m_p}$$

In a collision involving the parent ion of TCDD (m/z 322) and a molecule of target gas argon (40 amu) and a collision energy of 25 eV, the maximum amount of translational energy available for conversion to internal energy would be: 25 x 40/(40+322) = 2.76 eV.

Another significant variable affecting CID processes is whether the parent ion will undergo a single collision or multiple collisions with the target gas. An indicator of which conditions are present in an experiment is the cross section constant, σ, which relates the number of interactions, n_j, to the product of the number of incident particles, N, the number density of the collision gas, n, and the path length, L in the following equation (Cooks, 1978):

$$\sigma = n_j / NnL$$

The conditions in which n_j is linearly dependent on n and N are called single-collision conditions. After a point, n_j will not increase linearly with n and N, and a saturation effect will be observed. Past this point, multiple collision conditions exist. The parameter varied in this case is n, the number density of the gas. Generally, reduction of beam transmittance with collision gas by 50% or more is well within the region of multiple collisions (Todd, 1981).

Thus, the efficiency of formation and intensity of product ions in MS/MS depends on the collision energy, nature of the collision gas, and collision gas pressure (Charles, 1991).

Criteria for Mass Spectrometric Analysis and Identification of PCDD/Fs

In trace analysis of halogenated compounds (either HRMS or MS/MS), a number of criteria must be met to ensure the reliability of the data for identification and quantification. As discussed, two ions in the chlorine isotope cluster are monitored, and internal standards (usually ^{13}C-labeled in the case of PCDD/F analysis) are added to samples to permit quantification by isotope dilution. For chlorinated compounds, the measured ratio between the ions is compared to the theoretical ratio.

A compound with one Cl atom will actually contain both ^{35}Cl and ^{37}Cl. The mass spectrum of the molecular ion of this compound would contain at least two peaks, one ion containing ^{35}Cl (the M^+ ion) and one ion containing ^{37}Cl (the $(M+2)^+$). The ratio of the area of the two peaks will correspond to their natural isotopic abundance (approximately 3 to 1 ^{35}Cl to ^{37}Cl). The spectrum of compounds with an increasing number of Cl atoms will have a wider distribution of isotopic masses (M+4, M+6, etc.) and varying peak intensities. For example, the spectrum (as shown in Figure 1.20) of a tetra-chlorinated-dibenzo-*p*-dioxin (TCDD) will have its two most intense molecular ion peaks at m/z 320 (M^+) and m/z 322 ($(M+2)^+$). The ratio of M^+ to $(M+2)^+$ should be approximately 0.77. MS/MS analysis of PCDD/Fs involves monitoring the product ion produced from a loss of $CO^{35}Cl$. The isotope ratio between the product ions monitored depends upon two factors: the isotope ratio of the parent ions monitored and the probability that the parent ions will undergo the loss of $CO^{35}Cl$ as opposed to $CO^{37}Cl$. Using TCDD as an example,

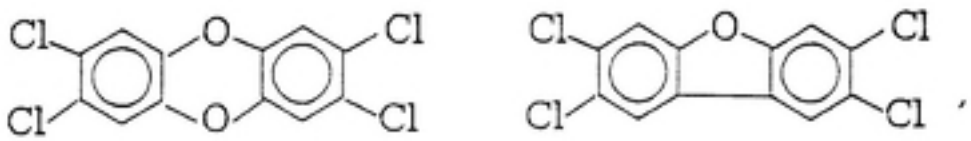

Figure 1.10. Stucture of 2,3,7,8-TCDD and 2,3,7,8-TCDF molecules, respectively.

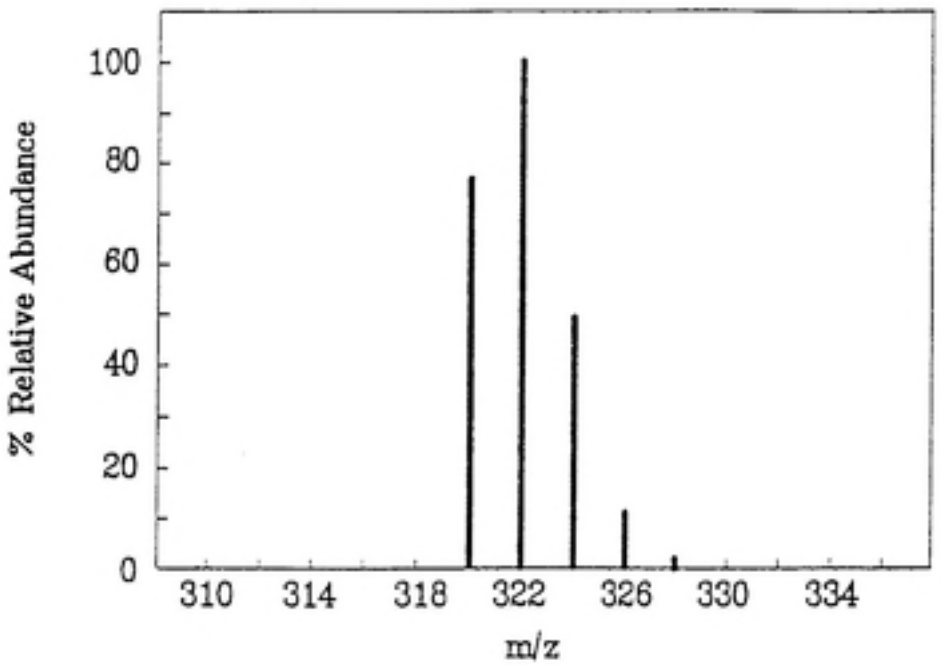

Figure 1.20. Mass spectrum of TCDD molecular ion showing chlorine isotope distribution.

imagine that M^+ and $(M+2)^+$ are passed into the collision cell of a mass spectrometer, and undergo losses of $CO^{35}Cl$. The ratio of the M^+ ($C_{12}H_4{}^{35}Cl_4$) ion to$(M+2)^+$ ($C_{12}H_4{}^{35}Cl_3{}^{37}Cl$), the parent ions, is 0.77. The probability that the M^+ ion (which contains only ^{35}Cl atoms) will lose $CO^{35}Cl$ is 1. The probability that the $(M+2)^+$ ion will lose $CO^{35}Cl$ is 0.75 because three of its four Cl atoms are ^{35}Cl. The theoretical isotope ratio for the resulting fragment ions (M^+ - $CO^{35}Cl$) and($(M+2)^+$ - $CO^{35}Cl$) can be calculated as follows:

$$\frac{(M^+)}{(M+2)^+} * \frac{Probability\ of\ (M^+ - CO^{35}Cl)}{Probability\ of\ ((M+2)^+ - CO^{35}Cl)}$$

which in this case becomes:

$$0.77 * \frac{1.0}{0.75} = 1.03$$

Isotope ratios (with certain error limits) are useful as indicators of compound identity and proper instrument tuning.

An additional criteria for identification of the analyte is relative retention time. The elution order of PCDD/Fs on DB-5 fused silica columns are well documented (Kleopfer, 1889). The convention used for congener identification is that the peak must appear between the elution times of the first and last eluting isomers for a congener group. The retention time of an analyte signal relative to that of the internal standard is used to identify specific isomers.

Quantification of PCDD/Fs by Mass Spectrometry

Quantification of PCDD/Fs by either HRMS or MS/MS involves use of the isotopic dilution method. This method requires the addition of a measured quantity of ^{13}C-labeled standards to the samples prior to the extraction and clean-up of the samples. These standards behave the identically to the unlabeled native compounds in the samples; they will elute from a GC column at the same time as the native compounds, and they will behave the same way in the mass spectrometer (except their masses will be higher due to the twelve ^{13}C atoms on the molecule). For the purposes of calculating the amount of native material in the sample, the magnitude of the signal of the native material is compared to the magnitude of the signal of the labeled material to determine the response factor (RF). The response factor is then fitted to a calibration curve consisting of the response factors of solutions of known concentration plotted versus concentrations. The calibration standards all contain the same concentration of internal (labeled) standards. The great benefit of the isotope dilution method is its accuracy and resistance to the variation in sample workup and instrument sensitivity. If losses of analyte occur during cleanup, a proportional loss of internal standard should also happen. As long as recovery is acceptable (as set by analytical method protocol) the signal of the analyte compared to the internal standard is considered reliable, and the response factor valid.

Specific Research in MS/MS Analysis of PCDD/Fs

Researchers in different laboratories have successfully demonstrated the ability of mass spectrometry/mass spectrometry to analyze environmental samples for

PCDD/Fs, to obtain quantitation values that are comparable to the results of samples analyzed by high resolution mass spectrometry, and to remove interferants that appear in HRMS analyses (Tondeur and Niederhut, 1987; Reiner, 1990; Charles and Tondeur 1990; Huang et al., 1991).

Detection limits of MS/MS instruments have been approaching those of HRMS instruments but are still higher. By measuring the height of the noise from a midpoint rather than from peak to peak, Reiner was able to report a signal to noise ratio of 10 to 1 on 500 fg standard of 2378-TCDD and was able to detect less than a picogram of all PCDD/F congeners using a Finnigan MAT TSQ70. Others have also obtained signal to noise ratios greater than 3 to 1 on a picogram of 2378-TCDD on different instruments (Charles and Tondeur, 1990). In general, the detection limits of MS/MS instruments are now within an order of magnitude or less than HRMS instrumentation. The S:N figure from Reiner is comparable to results obtainable by high resolution mass spectrometry. While from the efforts of these and other researchers, it appears that MS/MS is a complimentary analytical method to HRMS for trace analysis of environmental contaminants, the technique does present some challenges to researchers wishing to implement it as a standard analytical tool.

Interferences can still arise when using MS/MS, however. Others have reported the appearance of mass interferences in MS/MS analyses that were not apparent in HRMS analyses (Fraisse, 1989). Additional problems reported by others include large increases (2 to 1) in relative response factors over a 2.5-200 pg/μL concentration range (Huang, 1991). Also, other concerns when using MS/MS are

stability of the instrument and the reproducibility of response factors. Lastly, tuning conditions of the instrument have a great effect on the isotope ratios of the product ions detected. In general, variation in observed isotope ratios has been greater than the limits set for an HRMS method ($\pm$ 15%) (Reiner 1990).

In summary, MS/MS analysis of PCDD/Fs and other environmentally significant compounds shows promise as a quantitative analytical tool in certain situations. It is generally believed that the MS/MS technique surpasses others in analytical specificity, but it is not widely used owing to its lower sensitivity and reproducibility (Huang, 1991). Researchers have made progress in overcoming some of the operational issues regarding MS/MS. Questions regarding its performance, tuning parameters, and long-term utility need to be answered to develop a standardized methodology.

In this study a standard MS/MS is developed and its performance compared to HRMS. PCDD/Fs are used as the model compounds for this study because of their environmental significance. The findings of the study should benefit both researchers interested in analysis of PCDD/Fs and those interested in MS/MS applications in environmental analysis.

OBJECTIVES OF THE STUDY

The overall objective of the study was to evaluate MS/MS as a quantitative tool. The specific objectives were:

1. To determine the stability of the instrument over short term (1 day) and long term (months) use.

2. To determine the reproducibility of the analyte to the internal standard over time.

3. To define a tuning procedure for PCDD/F analysis.

4. To determine acceptable identification and quantitation criteria for an MS/MS method.

5. To compare quantitation values of PCDD/Fs in environmental samples by using MS/MS and HRMS.

6. To determine the accuracy and precision of the MS/MS method for PCDD/F analysis.

METHODS AND MATERIALS.

Standards preparation: Standard solutions of native and isotopically labelled polychlorinated dibenzo-*p*-dioxins and dibenzofurans were prepared by making serial dilutions of standard materials (2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDD and OCDF) obtained from Cambridge Isotopes Laboratory and Northrop Environmental Service ($^{13}C_{12}$ 2,3,7,8-TCDD, $^{13}C_{12}$ 1,2,3,7,8-PeCDD, $^{13}C_{12}$ 1,2,3,6,7,8-HxCDD, $^{13}C_{12}$ OCDD, $^{13}C_{12}$ 2,3,7,8-TCDF, $^{13}C_{12}$ 1,2,3,7,8 HpCDF). The final concentrations of these solutions contained 2.5, 10, 50, 200 and 1000 pg/μL of the native PCDDs and PCDFS and 100 pg/uL of the isotopically labelled materials (except ^{13}C OCDD which was at a concentration of 200 pg/μL)(Table 1). Standards were stored in capped volumetric vials and refrigerated when not in use. Stability of the standards was measured prior to use by comparing the response factor from a 35pg/uL NBS standard solution of 2,3,7,8-TCDD to the 2,3,7,8-TCDD response factor from the 50 pg/ul calibration solution to check that the difference between the response factors did not vary by what would be expected from instrumental error. In all congener groups except tetra, only one internal standard (either a PCDD or PCDF) was used for quantitation of both PCDDs and PCDFs dioxins and dibenzofurans, due to the availability of materials.

Table 2.1
Composition of the Standard Solutions Used to Construct Calibration Curves for High Resolution Mass Spectrometry and Mass Spectrometry/Mass Spectrometry

Compound	Concentration (Picograms/microliter)				
Unlabeled Analytes Solution	1	2	3	4	5
2,3,7,8-TCDD	2.5	10	50	200	1000
2,3,7,8-TCDF	2.5	10	50	200	1000
1,2,3,7,8-PeCDD	2.5	10	50	200	1000
1,2,3,7,8-PeCDF	2.5	10	50	200	1000
2,3,4,7,8-PeCDF	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDD	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDF	2.5	10	50	200	1000
2,3,4,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDD	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDF	2.5	10	50	200	1000
1,2,3,4,7,8,9-HpCDF	2.5	10	50	200	1000
OCDD	2.5	10	50	200	1000
OCDF	2.5	10	50	200	1000
Internal Standards					
13C12-2,3,7,8-TCDD	100	100	100	100	100
13C12-1,2,3,7,8-PeCDD	100	100	100	100	100
13C12-1,2,3,6,7,8-HxCDD	100	100	100	100	100
13C12-OCDD	200	200	200	200	200
13C12-2,3,7,8-TCDF	100	100	100	100	100
13C12-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100

Another standard was prepared for the collision energy experiments. This standard contained two more TCDD isomers (1,2,3,4-TCDD and 1,2,8,9-TCDD) at approximately 500 pg/μL of each compound.

Description of Instrumentation:

Research experiments were conducted on a VG70-250SEQ hybrid tandem mass spectrometer (VG Analytical, Altrincham, UK) with an EBqQ geometry (figure 2.10). The first mass analyzer (MS1) is a double-focusing mass spectrometer and MS2 is a quadrupole mass spectrometer. The collision cell is an RF-only quadrupole.

A high-sensitivity electron ionization source as supplied by the manufacturer was used in the instrument at a source temperature of 275°, electron energy of 34 eV and a filament emission current of 0.5 mA. Ions formed in the source were accelerated at a source potential of 8kV and focused by a series of lenses. The electric sector (E), or ESA (electrostatic analyzer), separates ions based on their kinetic energy. The magnetic sector (B) separates ions based on their mass/charge (m/z) ratio. For mass selection in selected ion recording experiments, the voltage into the magnet is stepped to correspond to a specific mass according to the fundamental equation for mass analysis in magnetic sectors ($m/z = B^2r^2c/2V$).

The quadrupole assembly, as mentioned, consists of two sets of quadrupoles and their lens assemblies. The first one, q, acts like a strong focusing lens and transports ions without mass analysis. It is an rf-only quadrupole and thus acts as a high pass filter. It also is the region where the collision induced dissociations occur. Dimensions of the cell are 3 cm in diameter and 10 cm in length. The mass analyzer quadrupole, Q, is an

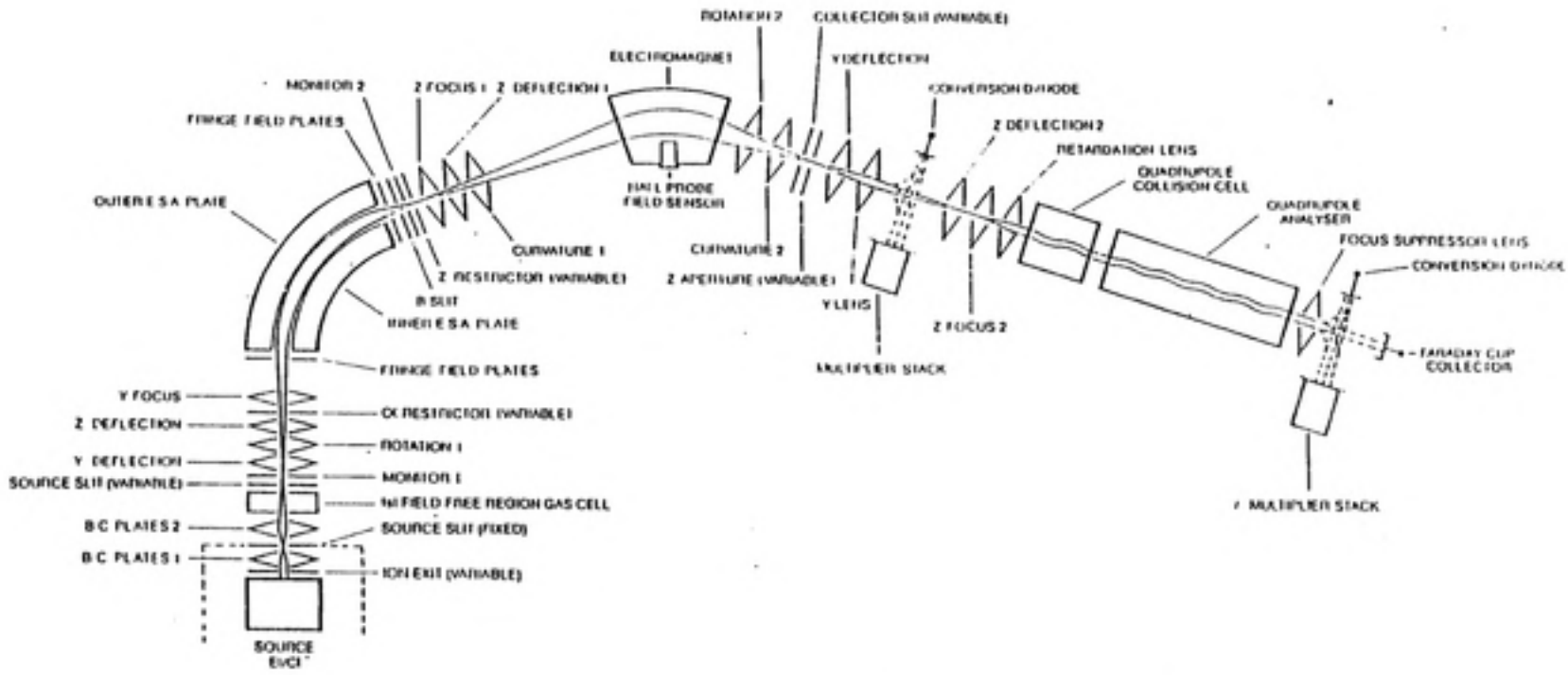

Figure 2.10. VG 70-250SEQ Hybrid Tandem Mass Spectrometer (EBqQ)

rf/dc quadrupole operating at a frequency of 920 kHz for a 3000 Da mass range with a radio frequency of $6kV_{p-p}$ and a maximum dc resolving voltage of 1000 V. When an ion enters Q with motion parallel to the z-axis, the applied rf and dc fields (which are perpendicular to the z-axis), cause the ion to undergo sinusoidal, periodic motion to the quadrupole axis. By selection of certain parameters, a given range of m/z values will possess a stable motion within the quadrupole and be transmitted to the detector. The range of stable m/z values depends on the rf/dc ratio.

The instrument interfaced with a VAX Station 2000 running OPUS version 1.5F software. All scan functions were controlled by the data system.

High-resolution gas chromatography: An HP5890 high-resolution gas chromatograph was interfaced to the mass spectrometer. A DB-5, 60-m-length, 0.25 mm-i.d. fused-silica capillary column (J&W Scientific) was used with a temperature program of 150° for 1 minute followed by a temperature rise of 20°/min. to 190° and 3°/min. to 300° for eight minutes. Prior to constructing a calibration curve, the elution windows of the tetra through octa PCDD/F congeners were set by analyzing a solution that contained the first and last eluting isomers for each PCDD and PCDF congener group at 100 pg/μL (Table 2.2). The volume injected into the GC was 1.0 μL. The split/splitless injection well was used, held at a temperature of 275°C with a purge valve switch on delay of 1.0 minute. A 2mm (inside diameter) glass injection port liner was used.

TABLE 2.2 RETENTION WINDOW MIX

CONGENER	ISOMERS FIRST ELUTING ISOMER	LAST ELUTING ISOMER
TCDD	1,3,6,8	1,2,8,9
TCDF	1,3,6,8	1,2,8,9
PeCDD	1,2,4,7,9	1,2,3,8,9
PeCDF	1,3,4,6,8	1,2,3,8,9
HxCDD	1,2,4,6,7,9/ 1,2,4,6,8,9	1,2,3,4,6,7
HxCDF	1,2,3,4,6,8	1,2,3,4,8,9
HpCDD	1,2,3,4,6,7,9	1,2,3,4,6,7,8
HpCDF	1,2,3,4,6,7,8	1,2,3,4,7,8,9

All compounds in retention window solution @ 100 pg/uL

High-resolution selected-ion-monitoring mass spectrometry: Experiments were performed on a VG70SEQ hybrid mass spectrometer described earlier operated in the selected ion recording mode with a resolving power of 10,000 (10% valley definition). Dwell times were 50 ms for the native and isotopically labelled PCDDs and PCDFs and 20 ms for the PFK lock mass. Delay times were 10 ms. The ions of two most abundant isotopes of each compound were monitored (see Table 2.3). (The isotopes monitored were either the M^+ and $(M+2)^+$ or the $(M+2)^+$ and the $(M+4)^+$ ions.)

MS/MS Selected Reaction Monitoring: Experiments were performed on a VG70SEQ hybrid mass spectrometer described previously operated in the selected reaction monitoring mode. Initial tuning of the instrument for MS/MS experiments was performed to optimize the intensity of the m/z 331 of PFK on MS1 at a resolution of 1000. This ion was then transmitted to MS2.

The collision energy and high mass analyzer ion energy zero settings were then examined. This inspection was done by first using the data system to set the collision energy to zero. A potentiometer on the collision energy high voltage board within the instrument was then adjusted so that single-ion signals were visible on the instrument oscilloscope. The collision energy was adjusted incrementally until beam height was maximized (usually 20-25 eV) and then the high mass analyzer ion energy was set to zero. Again, a potentiometer within the instrument was adjusted in the same manner described for the collision energy. The PFK was then eliminated and 2,3,7,8-TCDD was introduced into the source via a probe cup and the m/z 322 parent ion was directed

through MS1 into MS2 and the peak was optimized by adjusting the lenses for MS2. Care was taken not to change the ion energy setting for MS1, the ion repeller voltage of the source, or the system operating voltage (approximately 8 kV), as any of these changes would necessitate readjusting the collision energy and analyzer energy zero. The collision gas (argon) was bled slowly into the collision cell until an indicated pressure of $1x10^{-4}$ mbar was reached. This value correspond to about 80% attenuation of the beam as measured on the instrument oscilloscope. Refinements in the tuning of the instrument were made by adjusting the lenses a final time. The resolution of MS1 was changed from 1000 to 500 by opening the collector slit. For MS2, unit mass resolution was defined by measuring the base peak width of the m/z 257 and m/z 259 product ions from the parent m/z 322 monitored on the instrument oscilloscope. By definition, the width of each peak at the base and the space separating the two peaks were the same size to define unit mass resolution. The probe containing the TCDD was removed. PFK was reintroduced via the septum reservoir for calibration of the instrument and removed upon successful calibration. The two most abundant ion in the chlorine isotope cluster of each congener were monitored. Selected reaction monitoring of the $(M\text{-}CO^{35}Cl)^+$ daughter ions formed during collision induced dissociations was completed on MS2 set at unit mass resolution. The ions and transitions that were monitored are presented in Table 2.3. Dwell times were 40 ms for the native analytes and 10 ms for the isotopically labelled standards.

Sample preparation: A sediment sample was obtained from the Hudson River, New York. This sample was chosen because the sediment was known to contain PCBs,

Table 2.3 Composition and Masses of Transitions Monitored in the MS/MS Experiment[1]

Compound	Isotope Type	Exact Mass of Parent Ion	Mass of Product Ion	Transition Monitored
TCDF	$(M)^+$	303.9016	241	$C_{12}H_4{}^{35}Cl_4O \rightarrow C_{11}H_4{}^{35}Cl_3$
	$(M+2)^+$	305.8987	243	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO \rightarrow C_{11}H_4{}^{35}Cl_2{}^{37}Cl$
$^{13}C_{12}$-TCDF	$(M)^+$	315.9419	252	$^{13}C_{12}H_4{}^{35}Cl_4O \rightarrow C_{11}H_4{}^{35}Cl_3$
	$(M+2)^+$	317.9389	254	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO \rightarrow C_{11}H_4{}^{35}Cl_2{}^{37}Cl$
TCDD	$(M)^+$	319.8965	257	$C_{12}H_4{}^{35}Cl_4O_2 \rightarrow C_{11}H_4{}^{35}Cl_3O$
	$(M+2)^+$	321.8936	259	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2 \rightarrow C_{11}H_4{}^{35}Cl_2{}^{37}ClO$
$^{13}C_{12}$-TCDD	$(M)^+$	331.9368	268	$^{13}C_{12}H_4{}^{35}Cl_4O_2 \rightarrow {}^{13}C_{11}H_4{}^{35}Cl_3O$
	$(M+2)^+$	333.9339	270	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2 \rightarrow {}^{13}C_{11}H_4{}^{35}Cl_2{}^{37}ClO$
PeCDF	$(M+2)^+$	339.8957	277	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO \rightarrow C_{11}H_3{}^{35}Cl_3{}^{37}Cl$
	$(M+4)^+$	341.8567	279	$C_{12}H_4{}^{35}Cl_3{}^{37}Cl_2O \rightarrow C_{11}H_3{}^{35}Cl_2{}^{37}Cl_2$
PeCDD	$(M+2)^+$	355.8546	293	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2 \rightarrow C_{11}H_3{}^{35}Cl_3{}^{37}ClO$
	$(M+4)^+$	357.8516	295	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2 \rightarrow C_{11}H_3{}^{35}Cl_2{}^{37}Cl_2O$
$^{13}C_{12}$-PeCDD	$(M+2)^+$	367.8949	304	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2 \rightarrow {}^{13}C_{11}H_3{}^{35}Cl_3{}^{37}ClO$
	$(M+4)^+$	357.8516	295	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2 \rightarrow {}^{13}C_{11}H_3{}^{35}Cl_2{}^{37}Cl_2O$

[1]EXACT MASSES LISTED ARE THE SAME USED IN HIGH RESOLUTION EXPERIMENTS

Table 2.3 Composition and Masses of Transitions Monitored in the MS/MS Experiment (continued)

Compound	Ion Type	Exact Mass of Parent Ion	Mass of Product Ion	Transition Monitored
HxCDF	$(M+2)^+$	373.8516	311	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO \rightarrow C_{11}H_2{}^{35}Cl_4{}^{37}Cl$
	$(M+4)^+$	375.8178	313	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O \rightarrow C_{11}H_2{}^{35}Cl_3{}^{37}Cl_2$
HxCDD	$(M+2)^+$	389.8157	327	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2 \rightarrow C_{11}H_2{}^{35}Cl_4{}^{37}ClO$
	$(M+4)^+$	391.8127	329	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2 \rightarrow C_{11}H_2{}^{35}Cl_3{}^{37}ClO$
$^{13}C_{12}$-HxCDD	$(M+2)^+$	401.8559	338	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2 \rightarrow {}^{13}C_{11}H_2{}^{35}Cl_4{}^{37}ClO$
	$(M+4)^+$	403.8530	340	$^{13}C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2 \rightarrow {}^{13}C_{11}H_2{}^{35}Cl_3{}^{37}ClO$
HpCDF	$(M+2)^+$	407.7818	345	$C_{12}H^{35}Cl_6{}^{37}ClO \rightarrow C_{11}H^{35}Cl_5{}^{37}Cl$
	$(M+4)^+$	409.7789	347	$C_{12}H^{35}Cl_5{}^{37}Cl_2O \rightarrow C_{11}H^{35}Cl_4{}^{37}Cl_2$
$^{13}C_{12}$-HpCDF	$(M+2)^+$	417.8253	354	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO \rightarrow {}^{13}C_{11}H^{35}Cl_5{}^{37}Cl$
	$(M+4)^+$	419.8220	356	$^{13}C_{12}H^{35}Cl_5{}^{37}Cl_2O \rightarrow {}^{13}C_{11}H^{35}Cl_4{}^{37}Cl_2$
HpCDD	$(M+2)^+$	423.7766	361	$C_{12}H^{35}Cl_6{}^{37}ClO_2 \rightarrow C_{11}H^{35}Cl_5{}^{37}ClO$
	$(M+4)^+$	425.7737	363	$C_{12}H^{35}Cl_5{}^{37}Cl_2O_2 \rightarrow C_{11}H^{35}Cl_4{}^{37}Cl_2O$

Table 2.3 Composition and Masses of Transitions Monitored in the MS/MS Experiment (continued)

Compound	Ion Type	Exact Mass of Parent	Mass of Product	Transition Monitored
OCDF	$(M+2)^+$	441.7128	379	$C_{12}{}^{35}Cl_7{}^{37}ClO \rightarrow C_{11}{}^{35}Cl_6{}^{37}Cl$
	$(M+4)^+$	443.7399	381	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O \rightarrow C_{11}{}^{35}Cl_6{}^{37}Cl$
OCDD	$(M+2)^+$	457.7377	395	$C_{12}{}^{35}Cl_7{}^{37}ClO_2 \rightarrow C_{11}{}^{35}Cl_6{}^{37}ClO$
	$(M+4)^+$	459.7348	397	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2 \rightarrow C_{11}{}^{35}Cl_6{}^{37}Cl_2O$
$^{13}C_{12}$-OCDD	$(M+2)^+$	496.7780	406	$^{13}C_{12}{}^{35}Cl_7{}^{37}ClO_2 \rightarrow {}^{13}C_{11}{}^{35}Cl_6{}^{37}ClO$
	$(M+4)^+$	471.7750	408	$^{13}C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2 \rightarrow {}^{13}C_{11}{}^{35}Cl_6{}^{37}Cl_2O$

compounds that can interfere with the analysis of PCDDs and PCDFs. Samples of fly ash from a Chicago incinerator were also obtained. In the laboratory, the sediment samples (20 g.) and a laboratory blank were fortified with a toluene solution that contained the isotopically labeled internal standards. The sediment was first soxhlet-extracted in 400 ml isopropanol for 16 hours and then soxhlet extracted in 400 ml toluene for 15 hours. The extracts were combined and exchanged to hexane. The PCDDs and PCDFs were then isolated by passing the sample extracts through a silica gel column a basic alumina column, and finally a carbon/celite column eluted with a variety of solvent mixtures, the last being toluene (see figure 2.20).

Reagents: Solvents used were Fisher Optima grade hexane, toluene, dichloromethane, isopropanol, benzene, cyclohexane, and ethyl acetate. Silica gel was Biosil-A (100-200 mesh from Bio-Rad Laboratories. Basic alumina (80-200 mesh, Fisher Scientific) was also used. The carbon/celite mixture was composed of Carbon AX-21 (Anderson Development Co.) and celite 545 (Fisher Scientific).

Column Chromatography Preparation: The silica gel was activated for use by heating in a vacuum oven at 180°C for 90 minutes. After cooling, the material was washed with methanol and dichloromethane, placed in the vacuum oven and heated up to 180°C to dry it. The activated Biosil-A was stored in a desiccator. The column was prepared by plugging the tip of a 25 ml glass pipet with hexane washed glass wool and adding 8 g. of silica gel. A 1 cm layer of sodium sulfate was added on top of the silica

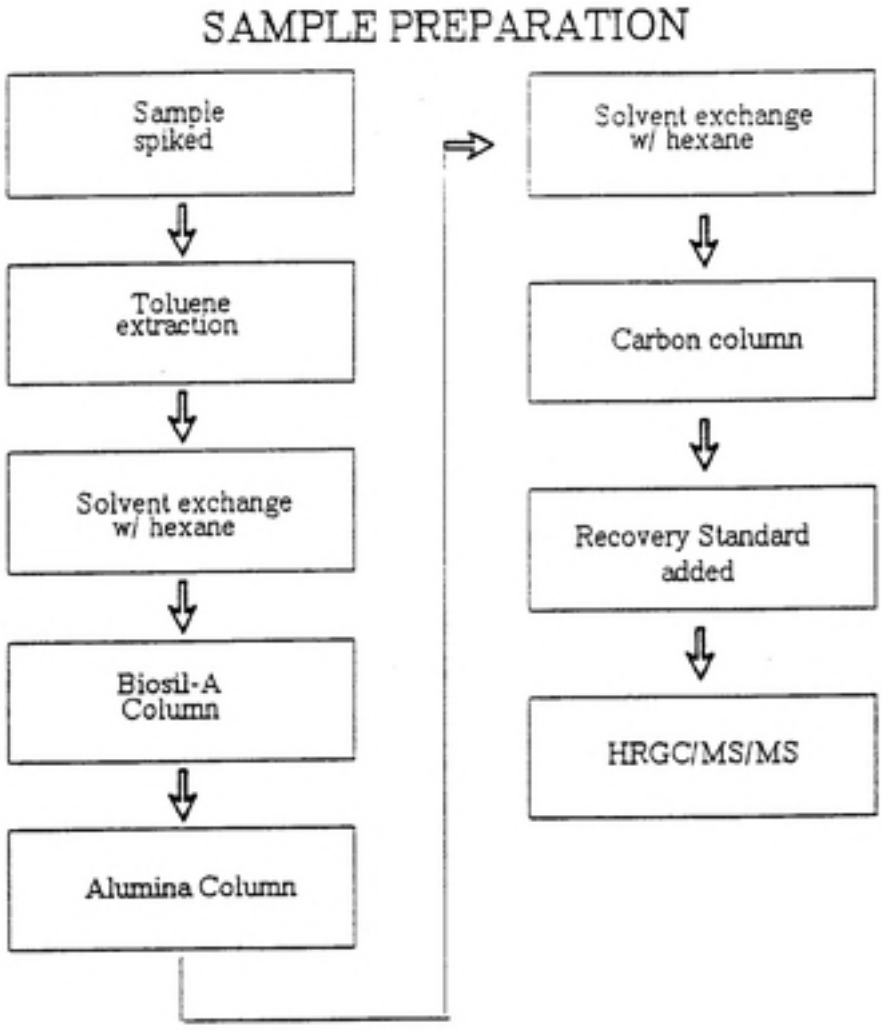

Figure 2.20. Flowchart of clean-up procedure used to prepare samples for mass spectrometric analysis.

gel. Before use, the column was washed with three 10 ml aliquots of hexane. The sample was then loaded on the column, followed by two 5 ml hexane rinses of the sample container and 120 ml of hexane. This eluent was collected and rotary evaporated to approximately 5 ml and stored in a sealed test tube until the next step in the procedure was performed.

Basic alumina was activated by heating overnight in a vacuum oven at 200°C for ten hours. After cooling, the material was used right away or stored in a desiccator. Alumina columns were prepared by plugging the tip of a 250 ml reservoir column with hexane washed glass wool and putting in 50 g of activated alumina. A 1 cm layer of sodium sulfate was put over the top of this material. The sample was then loaded on the column, the sample container was rinsed three times with hexane (5 ml), and the rinses were loaded on the column. Then, 250 ml of hexane were added to the reservoir. The eluent was collected and disposed of properly. The column was then eluted with 250 ml of the 35%dichloromethane/65% hexane solution. Upon collection, this eluent was rotary evaporated to a volume of 10ml and then solvent exchanged with hexane. The extract was again evaporated down to about 2 ml final volume for treatment with the carbon/celite column.

The carbon column was made from a 2 ml glass pipette packed with a carbon/celite mixture. The mixture was prepared by mixing 5.35 g of Carbon AX-21 with 62 g of celite 545. The column was plugged at one end with glass wool and filled 2.5 cm with the carbon/celite mixture. A glass wool plug was placed on the top end of the mixture as well. Three solvent mixtures of the following compounds were prepared:

a 50/50 (by volume) solution of benzene and ethyl acetate, a 50/50 (by volume) solution of dichloromethane and cyclohexane, and a 50/50 (by volume) of dichloromethane and hexane. Prior to putting the sample on the column, the column was rinsed with 2ml of benzene/ethyl acetate, 1 ml of dichloromethane/cyclohexane, and 2 ml of hexane. The sample was placed on to the column, and the sample container was rinsed with 2 ml of dichloromethane/hexane and 2 ml of benzene/ethyl acetate. Both rinses were added to the column. When the solvents had passed through the column, the column was inverted and the PCDD/Fs were eluted with 10 ml toluene. The sample extracts were then evaporated under a flow of nitrogen to 1-3 μL. 50 μL of recovery standard solution (50 pg/μL of ^{13}C-1,2,3,4 TCDD and ^{13}C-1,2,3,7,8,9 HxCDD) were then added. The final volume of the extract was measured, and the extracts were stored in a refrigerator until analyzed.

For comparison of HRMS and MS/MS methods, three aliquots of fly ash from a Chicago municipal incinerator and laboratory blank were prepared. The fly ash samples were soxhlet extracted in toluene for 16 hours. The three sample extracts were analyzed by MS/MS; two were analyzed by high resolution mass spectrometry. The sample extracts were then quantified for total PCDD/PCDF content (congeners tetra through octa). For determination of accuracy, a fourth aliquot of the fly ash was spiked with 150μL of toluene solution containing 50 pg/μL of the 17 native standards listed in addition to the ^{13}C-labeled internal standards.

METHOD FOR QUANTIFYING PCDD/Fs IN SAMPLES

Samples were analyzed for PCDD/Fs by HRMS and MS/MS. The quantitation procedure was similar for each method. Prior to analysis of the samples with the mass spectrometer, a four point calibration curve was constructed (2.5, 10, 50, and 200 pg/μL calibration standards used). Signal responses (area) for both the native and internal standards were measured so that response factors (RFs) could be calculated. The response factor ($RF = A_A/A_{IS}$ where A_A and A_{IS} are the measured signal response for the analyte and internal standard, respectively) for each calibration point was plotted versus concentration. The equation of the line was derived by linear regression ($y = Mx + b$ where y is the response factor, x the concentration, M is the slope and b is the intercept.). A calibration curve was made for each PCDD and PCDF congener group. The samples were then analyzed and the response factors were calculated for each congener group and specific isomers where possible. These response factors (y) were put into the appropriate calibration curve line equation (based on compound and congener group) to yield concentration values for the sample extracts.

ANALYSIS OF SAMPLES FOR IDENTIFICATION OF INTERFERANTS

Comparison of the results from sample analysis by HRMS and MS/MS showed the presence of some type of material that prevented quantitation of the PeCDDs in the sediment samples. Two types of experiments were conducted to identify the interferants. The sample was analyzed by operating the mass spectrometer in the full scan mode (mass range 500 to 50 amu) at a mass resolution of 1000. Another experiment was performed by high resolution (10000 at 10% valley definition) selected ion recording. In this

experiment, ions in the mass spectra of hexachlorinated biphenyl were monitored (see Table 2.4 below). The same instrument conditions (resolution, dwell times, GC column and temperature program) used for the HRMS analysis for PCDD/Fs were used for this experiment.

Table 2.4
Ions Monitored for HRMS Analysis of Hexa-PCB

Ion Type	Isotope	Exact Mass
Parent	M^+	357.844
Parent	$(M+2)^+$	359.8416
Parent	$(M+4)^+$	361.8386
Fragment	$(M-Cl)^+$	322.8755
Fragment	$(M+2-Cl)^+$	324.8726
Fragment	$(M-2Cl)^+$	287.9067
Fragment	$(M+2-2Cl)^+$	289.9037

Studies on the Linearity and Reproducibility of the Relative Response Factors (RRFs):

Six calibration curves were constructed by measuring the response of the analyte (2.5, 10, 50, 200, 1000 pg/uL) to the internal standard (100 pg/uL). These analyses were completed over a period of 8, 24, and 92 hours, initially. Another curve was run at a 3 and a 7 month interval with the same standards used to construct the first calibration curve. After each curve was completed, the relative response factors (RRFs) and response factors (RFs) were calculated for each of the isomers in the calibration solutions. The RRFs were calculated by using the following equation:

$$RRF = \frac{A_A * C_{IS}}{A_{IS} * C_A}$$

where A_A and A_{IS} are the combined signal peaks for the two analyte channels and ^{13}C-labeled internal standard channels, respectively. C_A and C_{IS} are the concentrations of the analyte and internal standards, respectively. The stability of the instrument to perform quantitative measurements and the linearity of the response of the analyte to the internal standard was determined by calculating the mean, standard and percent relative standard deviation of the RRFs. No re-tuning or calibration of the instrument was performed among the first analyses and those obtained 92 hours later. The last two calibration curves were constructed by following the MS/MS tuning protocol and analyzing the same calibration standards used in the first curves. The instrument was used for other types

of analyses and experiments during the time between the construction of the last two calibration curves.

Collision Energy Experiments

Earlier experiments by workers in this laboratory suggested a collision energy optimum of 20 eV for the formation of $(M - COCL)^+$ product ions from PCDD/F parent ions. To determine the long term reproducibility of earlier work regarding collision energy optima for the $(M - COCl)^+$ fragmentation, and to investigate the possibility of collision energy optima differences between isomers, a standard solution containing PCDD/F tetra through octa isomers mentioned previously was analyzed by the following method. The 500 pg/μL solution was introduced into the instrument via the gas chromatograph. Under the selected reaction monitoring conditions described previously, the collision energy was stepped from 10 to 60 eV in 10 ev increments. These experiments were performed at four month intervals. The signal response (area) of each analyte was measured at each collision energy level and plotted to determine the maxima. During the second set of experiments, the standard solution was analyzed first under normal tuning conditions, and again after tuning the instrument on a higher mass congener than TCDD (the tuning material was OCDD) to investigate the possibility of tuning effects on collision energy optima.

Additional experiments to examine the effects of collision energy and total product ion formation were conducted to explain observed differences in the shapes of collision energy curves for PCDDs and for PCDFs. Individually, 2,3,7,8-TCDD, 2,3,7,8-TCDF, OCDD, and OCDF were introduced into the ion source of the instrument via direct

probe. A single m/z ratio for each compound was monitored in MS1, the collision energy was stepped from 10 to 80 eV in 10 eV increments, and MS2 was operated in the full scan mode over a mass range of 500 to 50 daltons. A comparison between the intensities and make up of observed product ions for PCDDs and PCDFs was then made.

Data Analysis:

Sample quantitation data analysis (mean value, standard deviation, and linear regression calculations) was performed on Lotus 1-2-3 spreadsheets. The statistical comparison of quantitation data from HRMS and MS/MS was performed on Systat (t-tests) and SAS (univariate approach to repeated measures analysis).

RESULTS AND DISCUSSION.

Collision Energy

Earlier work on the same instrument by Charles and Marbury determined that 20eV was the collision energy which provided optimum formation of the $(M - COCl)^+$ ion from PCDD/F. Repeated investigations yielded a different value from the earlier work (perhaps due to slight changes in instrument performance over a year's time). The optimal collision energy for 2,3,7,8-TCDD was 25-30 eV in the latest studies. The maxima for all congeners studied was also in this range (Table XX). Additional work by Charles and Marbury indicated no significant differences in collision energy maxima between isomers. The two follow up studies conducted later led to the same conclusion (Figures 3.00 and 3.05). Reiner et. al., however, reported a range of collision energy maxima between the 1234, 1368, 1278, and 2378 isomers of TCDD of 7 eV, with 2378 TCDD having the highest CE maximum at 25 eV. Furthermore, the signal responses of the other three isomers at a CE of 25eV were within 20% to 30% of their maximum values obtained at their optimal collision energies. While these findings differ from our results, the overall impact of slight differences in collision energy maxima, if actual, is small. Reiner commented that the observed 20%-30% difference in signal strength of the other TCDD isomers might lead to relative response factors influenced by collision energy setting. Experimental results from this study regarding the accuracy of the method suggest that a $\pm$ 30% difference from actual quantitative value is within

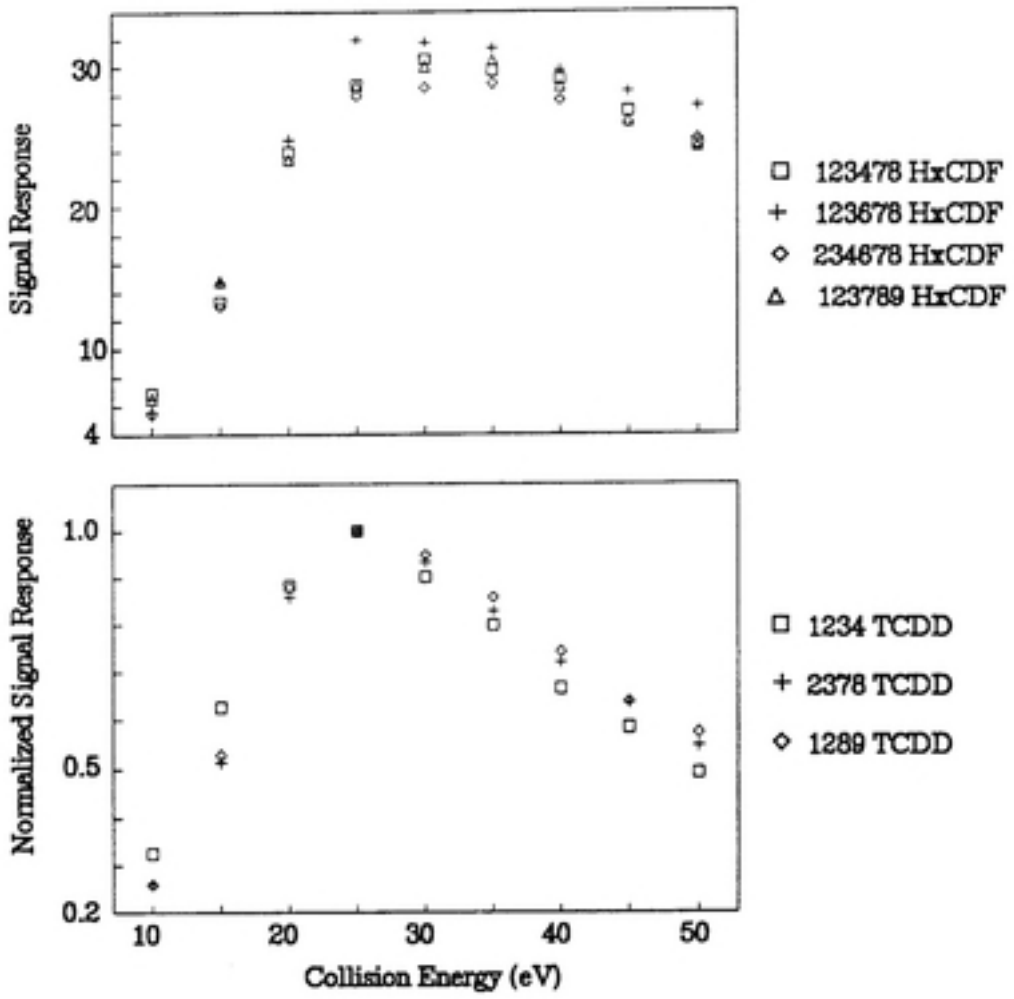

Figure 3.00. Effect of collision energy on the formation of $(M - COCl)^+$ product ions of different TCDD and HxCDF isomers.

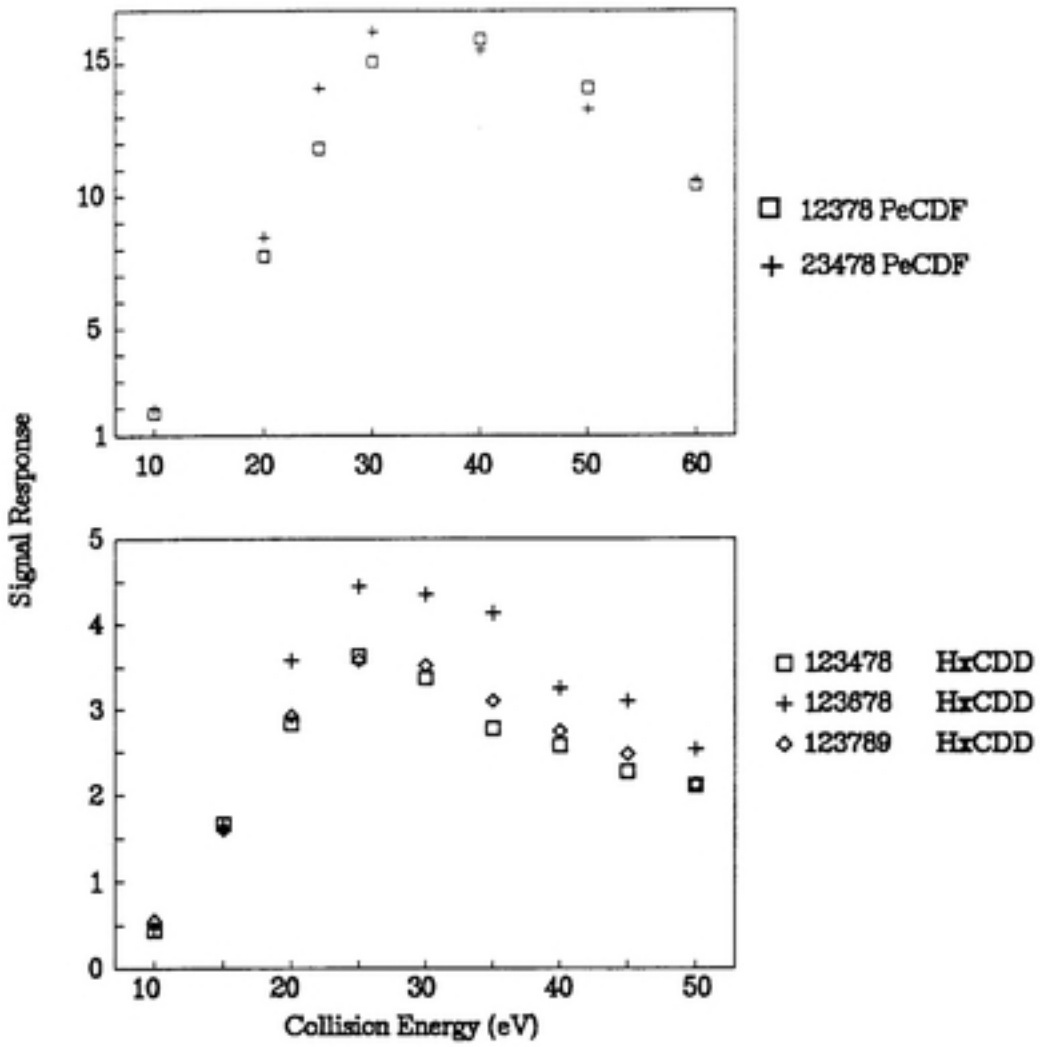

Figure 3.05. Effect of collision energy on the formation of $(M - COCl)^+$ product ions of different HxCDD and PeCDF isomers.

analytical error. Thus, the reported differences would not greatly influence the quantitative method. Variations in the MS/MS tuning procedure (using OCDD as a tuning compound instead of TCDD) did not change the maxima either. As a group, PCDFs exhibited a broader maxima for collision energy than PCDDs (10-15 eV vs. 5-10eV), but both have collision energy maxima in the same 25-30eV range (Figures 3.10-3.30). A 5 eV to 10 eV collision energy maxima range is comparable to what others have reported (Reiner, et al.). Based on this information, the recommendation is to perform collision energy optima experiments using a solution containing tetra through octa isomers that have chlorine atoms in the 2,3,7,8 positions prior to beginning MS/MS analyses, and then to check the results periodically (relative to usage) to observe if such analyses are to be performed on a continuing basis.

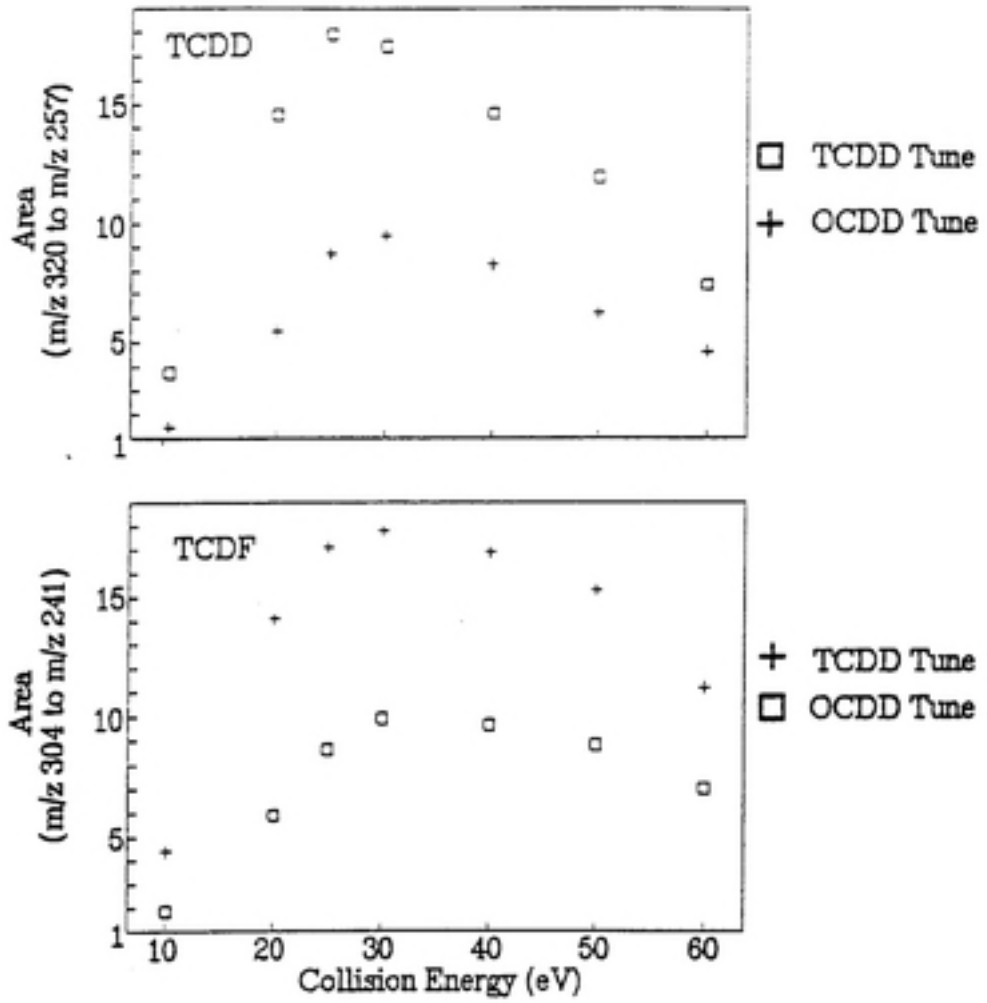

Figure 3.10. Effect of collision energy and instrument tuning material on formation of $(M - COCl)^+$ product ions of 2,3,7,8-TCDD and 2,3,7,8-TCDF.

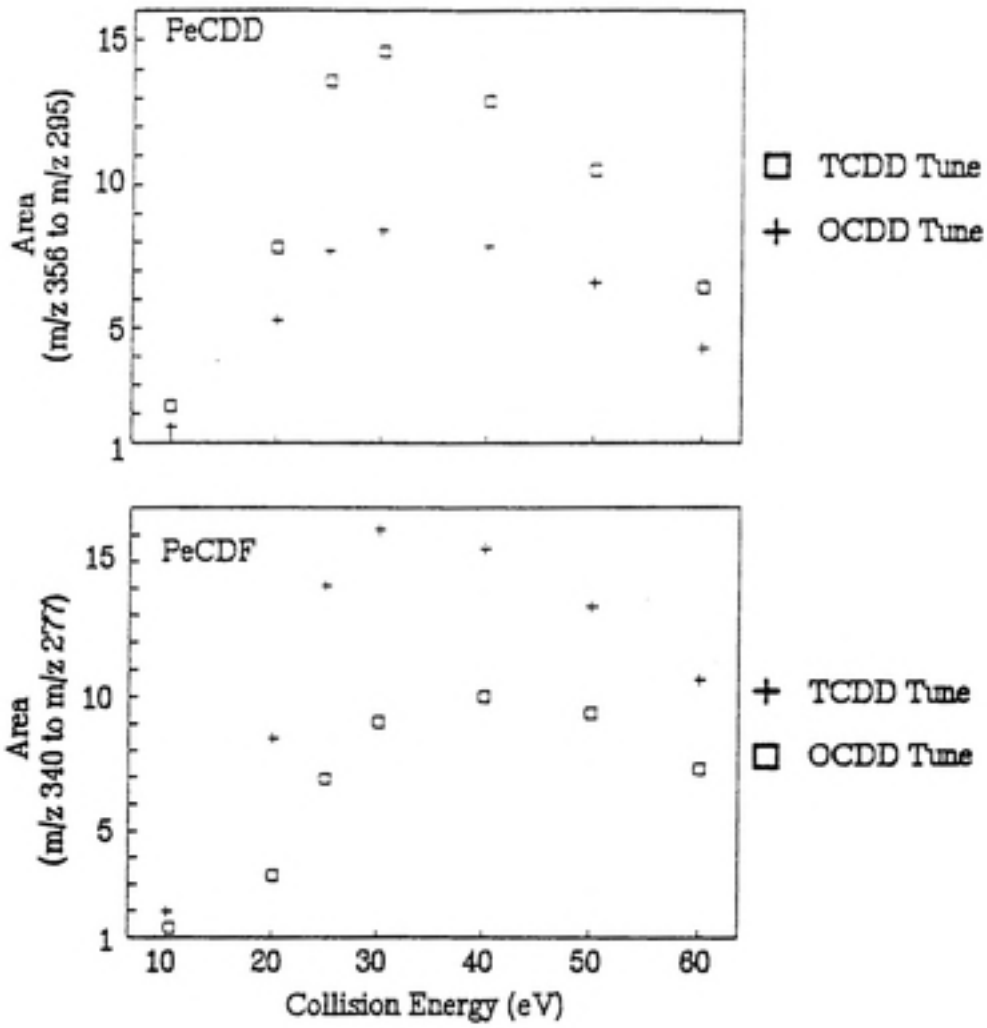

Figure 3.15. Effect of collision energy and instrument tuning material on formation of $(M - COCl)^+$ product ions of 1,2,3,7,8-PeCDD and 1,2,3,7,8-PeCDF.

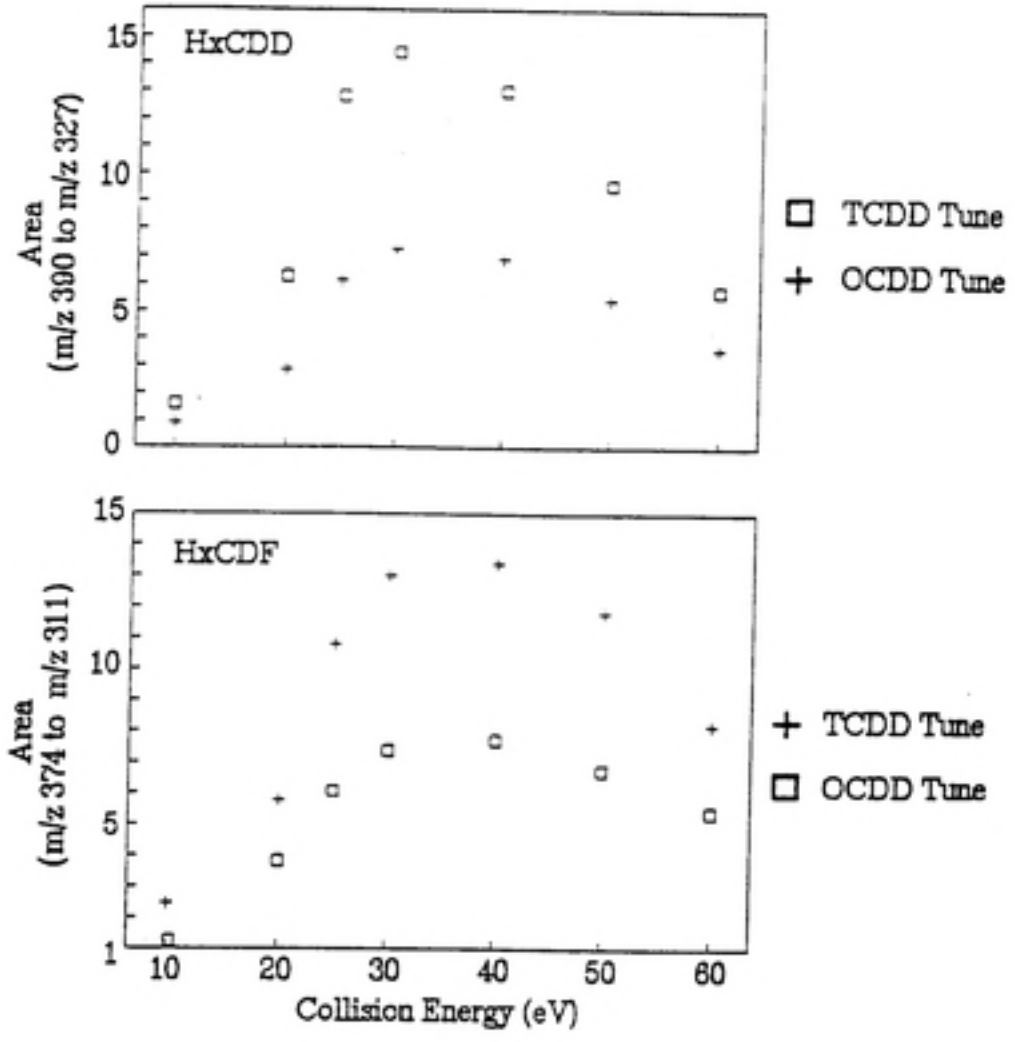

Figure 3.20. Effect of collision energy and instrument tuning material on formation of $(M - COCl)^+$ product ions of 1,2,3,7,8,9-HxCDD and 1,2,3,7,8,9-HxCDF.

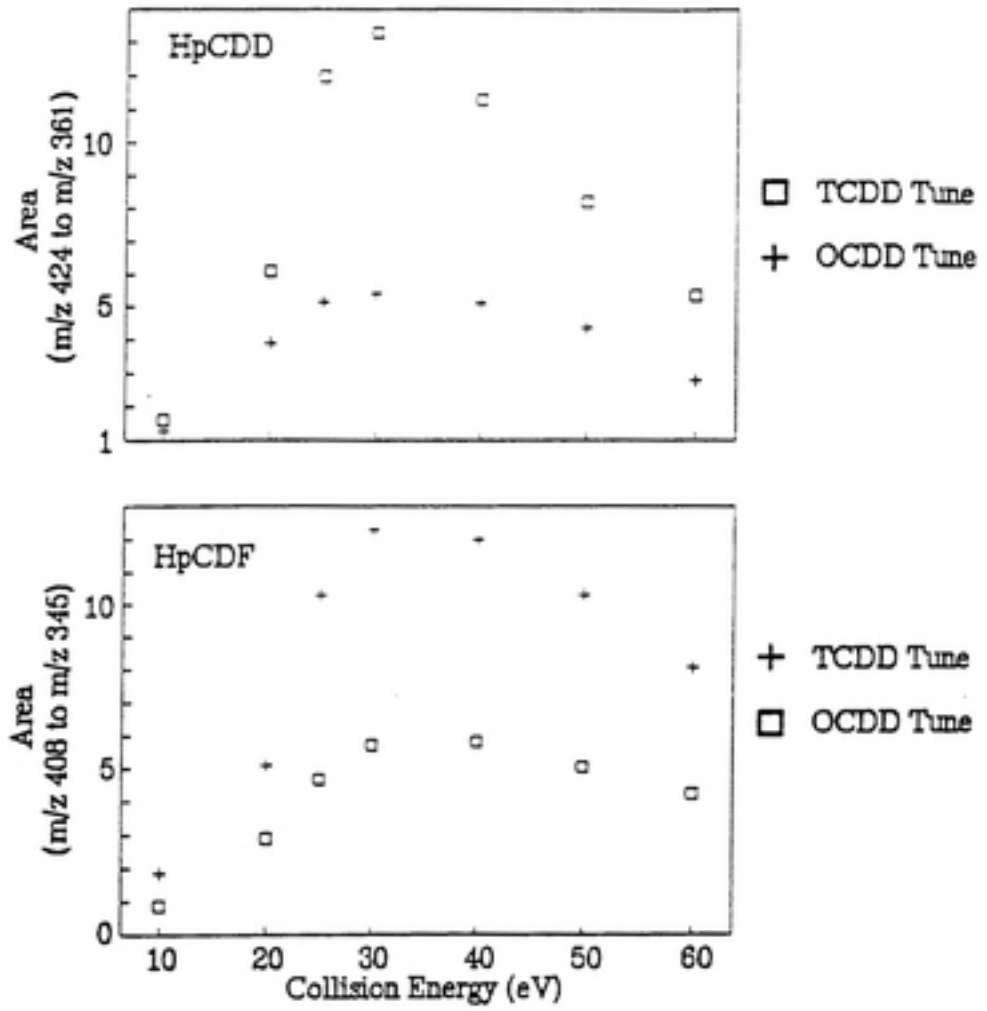

Figure 3.25. Effect of collision energy and instrument tuning material on formation of $(M - COCl)^+$ product ions of 1,2,3,4,6,7,8,-HpCDD and 1,2,3,4,6,7,8,-HpCDF.

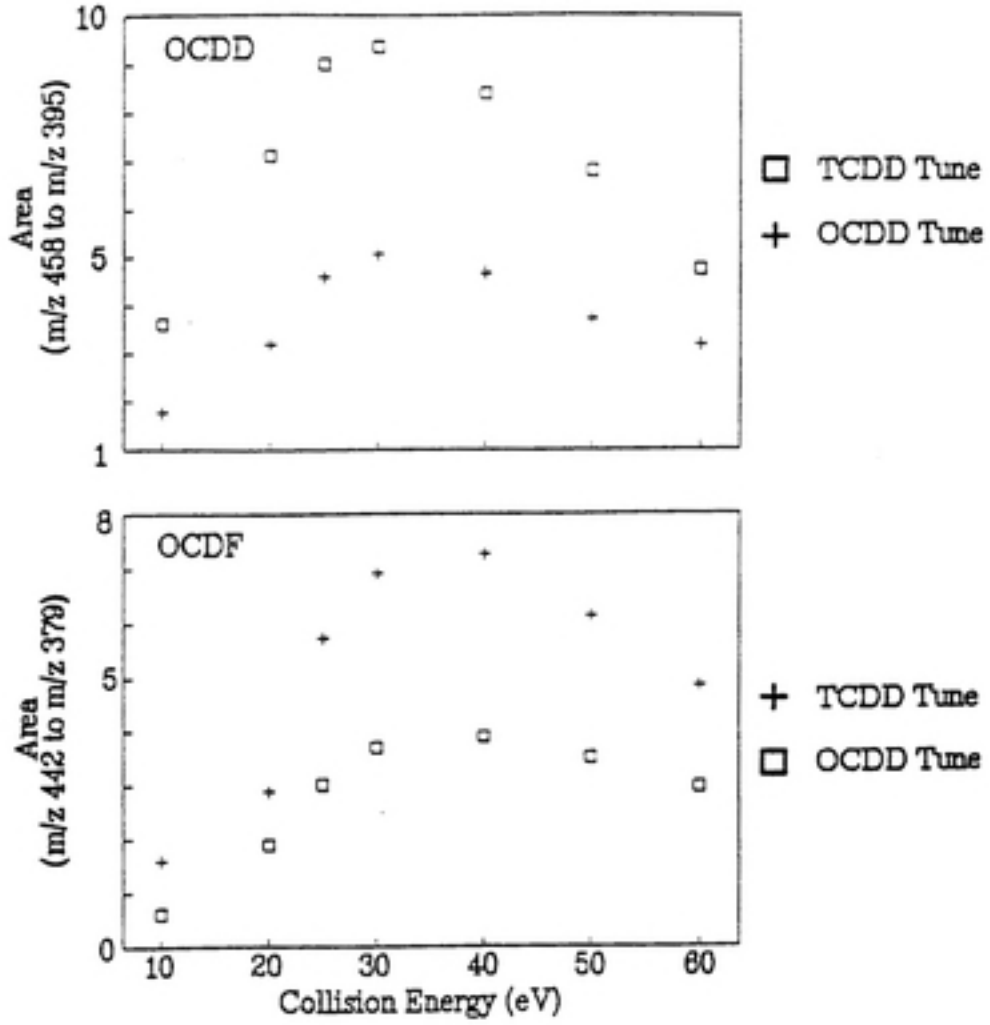

Figure 3.30. Effect of collision energy and instrument tuning material on formation of $(M - COCl)^+$ product ions of OCDD and OCDF.

From the product ion scan experiments of TCDD, TCDF, OCDD and OCDF, graphs of normalized product ion intensity versus collision energy were prepared in an effort to explain the broader collision energy maxima observed for PCDFs than PCDDs. The original SRM experiments were repeated three times, with the result being the same each time, leading to the conclusion that the difference in the shape of the collision curves is real and not an artifact. While the same difference was not visible from the full scan data, enough information was available to suggest explanations for the differences observed between PCDDs and PCDFs in the SRM experiments.

The daughter MS/MS spectrum of polyatomic parent ion is determined by the internal energy of the parent ion, the rate constants for each possible dissociation, and the time during the network of reactions is allowed to occur (Busch, 1988). Instrumental limitations make a determination of absolute characteristics impossible. For example, the measured appearance energy of fragment ions in an instrument will be higher than the thermochemical value (E_o) because internal energy is imparted to the ions as they are formed in the source (this appearance difference is known as a "kinetic shift" (McClafferty, 1980). Also, in most MS/MS instruments, ions typically have a microsecond after the activation reaction to dissociate before they reach the next stage of mass analysis, imposing a selection of detected daughter ions by rate constant (Busch, 1988). However, the purpose of the experiments was to get information for a comparison of PCDD and PCDF ions in the mass spectrometer, so the assumption is made that the instrumental factors influencing the daughter ion spectra of one group will apply equally to the other. Any discussion of rate constants and other factors relating

to ion dissociation will take place within the context of the specific instrumental system used in these experiments.

The normalized parent ion and the three most intense product ion intensities were plotted against collision energy (the other product ions were excluded because they contributed less than %5 of the total ion current) (figures 3.31-3.34). For both TCDF and OCDF, the only ions that contribute more than 15% to the total ion current are the parent ion and the $(M\text{-}COCl)^+$ ion. The TCDD and OCDD product ion scans show that the $(M\text{-}2COCl)^+$ ion formation is a large contributor to the TIC (up to 30 % past 20eV). An explanation of the difference between the shapes of the collision energy curves for PCDDs and PCDFs can be formed by examining the approximate form of the Rice-Ramsperger-Kassel-Marcus (RRKM) theory equation:

$$k(e) = v\,[\,(e-e_0)^{(n-1)}\,]/e$$

where k(ϵ) is the internal energy dependent rate constant, ϵ is the internal energy of the ion, ϵ_0 is the critical energy for a reaction, n is the number of oscillators (degrees of freedom), and v is the ratio of the product of the vibrational frequencies of the activated complex to that of the parent ion (Busch, 1988). The probability that a dissociation reaction will occur is dependent upon a combination of critical energy requirements and steric requirements if the dissociation involves rearrangement (McLafferty, 1983). PCDF ions, having undergone a COCl loss (and thus changing the variables in the rate

constant equation), may form a relatively stable complex that has less inclination to fragment further as readily in the given conditions as a PCDD undergoing a COCl loss. It may be that the other dissociation pathways available to the $(M - COCl)^{+}$ ions of PCDFs have lower rate constants than the first significant fragment loss due to a decrease in the number of oscillators (degrees of freedom left in the ion) which would exponentially decrease rate constants for other dissociations. Whether the PCDF ions do not form another major fragment ion because of a high critical energy required to do so or due to steric restraints is not clear. It may be a combination of both factors. The PCDD ions have the loss of an additional COCl loss available as a dissociation pathway and more degrees of freedom within the ion. Thus, as the internal energy of the PCDD ions increases as collision energy increases, the loss of 2COCl becomes a favorable dissociation that competes with the loss of COCl loss. The PCDF ions apparently do not have the possibility of experiencing a significant competing dissociation reaction within this collision energy range and therefore sustain the $(M-COCl)^{+}$ ion production at a higher intensity over a broader collision energy range than PCDD parent ions. The data simply show that, past 20 eV, $(M-2COCl)^{+}$ loss becomes a significant dissociation pathway for PCDDs. An exact corresponding decrease in $(M-COCl)^{+}$ loss in the 30-60 eV range was not discernable. This may be due to experimental or instrumental limitations and perhaps additional experiments might show this relationship more clearly.

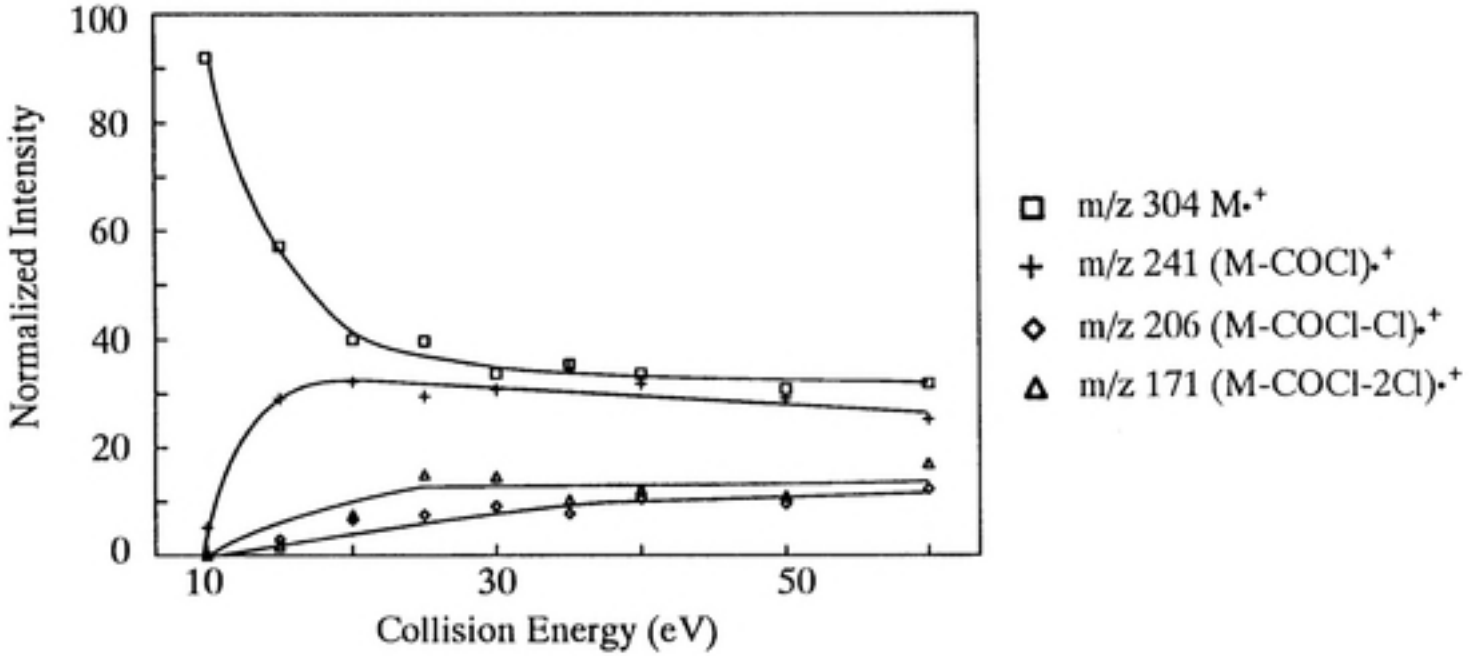

Figure 3.32. Effect of collision energy on major product ion formation from 2,3,7,8 TCDF parent ions.

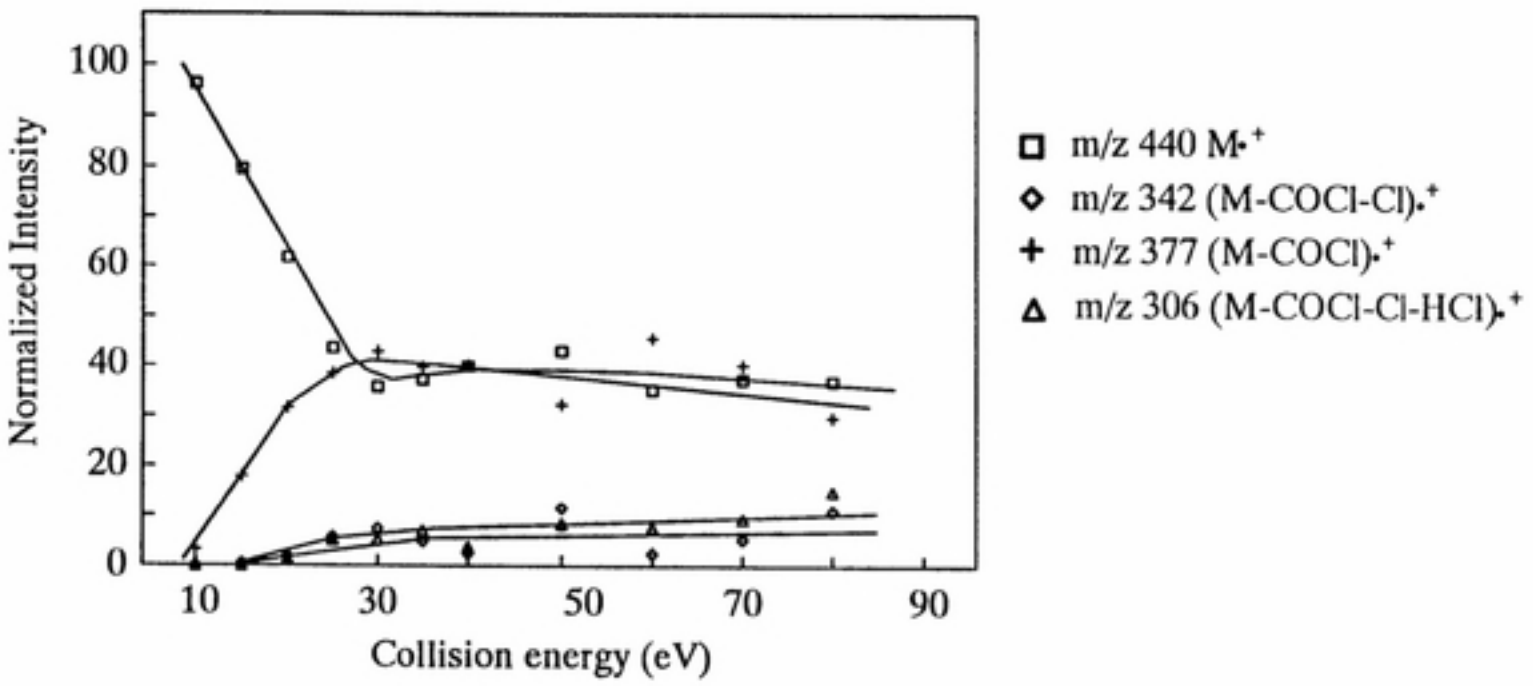

Figure 3.34. Effect of collision energy on major product ion formation from OCDF parent ions.

Calibration: The linearity in the response of the analyte to its concentration over a 2.5 pg/μL to 1000 pg/μL concentration range was made apparent by RRFs that had less than an 20 % relative standard deviations (%RSD). Overall, a relative percent standard deviation of less than 20% was obtained among the relative response factors for each of the 17 congeners. On the first day of analysis, the percent relative standard deviation among the RRFs ranged from 8-35% with a mean RSD of 14%. Analysis of the standard solutions after 8 and 24 hours, provided similar results. For these calibration curves, the relative standard deviations of the relative response factors ranged from 4-39% and 4-22% with mean values of 14% and 15%, respectively. It appears that the instrumental conditions changed during the 72 hours that the mass spectrometer was allowed to remain idle because a 41% RSD was observed for the OCDD and OCDF congeners, and an increase in the %RSD of the average RRFs for most other congeners was observed. The fact that the retention window mixture analyzed before this last curve appeared normal, with good isomer separation and peak shape, reinforces the conclusion that changing instrumental conditions were responsible for the increase in RRF variability, rather than the gas chromatography. The linearity of the response and the reproducibility of the response factors was again demonstrated when the analyses were performed after 3 months and 7 month intervals. During the period that elapsed the instrument was used for other type of mass spectrometric analyses that included the use of high-resolution and fast-atom bombardment mass spectrometry. The RRFs from these calibration curves were calculated and then analyzed for variability. The curves from the 3 month point had RRFs whose %RSDs varied from 6% to 13%. For the curves

made 7 months later the %RSD ranged from 4% to 17%. A tabular representation of the data shows the trend of increasing variation in RRFs over the first 92 hours and then a decrease in variation 3 and 7 months later after re-tuning. The compiled results of these calibration curves are in Table 3.1. The ability to perform quantitative measurements upon retuning and recalibrating the instrument was thus demonstrated. These results are different than those reported Huang et al., 1991. These investigators reported a concentration dependence in the RRFs obtained by MS/MS analyses of standard materials that contained the analytes in concentration ranges from 2.5-200 pg. Plots of the response of representative isomers from each congener group are presented in Figures 3.36A-3.36E to demonstrate the change in the distribution of individual RRF values around the mean RRF value made from all the curves. Ideally, all of the RRFs should have the same value. These plots show the general trend of a wider distribution of RRFs as the time from the initial tuning increases and then a decrease in the RRF variability after each re-tuning. The correlation coefficients obtained by regression analysis of all the calibration curves (2.5-1000 pg/uL) were 0.875 for 2,3,7,8-TCDD, 0.949 for 2,3,7,8-TCDF, 0.983 for 1,2,3,7,8-PeCDD and 0.911 for 1,2,3,7,8 PeCDF. For comparative purposes the correlation coefficients for curves resulting from the analysis of the 2.5-200 pg/uL standards were also calculated. These correlation coefficients were 0.886 for 2,3,7,8-TCDD, 0.952 for 2,3,7,8-TCDF, 0.965 for 1,2,3,7,8-PeCDD and 0.903 for 1,2,3,7,8-PeCDF. The correlation coefficients from linear regression calculations on calibration curves made on other congeners (hexa through octa PCDD/Fs) were all above 0.90. Thus, we observed linear responses over

the concentration range of 2.5-200 pg/uL and 2.5-1000 pg/uL were observed thereby enabling accurate quantitation by using mass spectrometry/mass spectrometry.

To determine the reproducibility of the response of the analyte to its internal standard, the RRFs at one concentration value 5 curves were averaged. The point from the fourth curve was omitted because it demonstrated a loss of linearity after 92 hours without any retuning. The RRFs of the 50 pg/μL point for 2,3,7,8-TCDD from each of the 5 different curves, when averaged, show a %RSD of 20% (Table 3.2). All the congener groups had a %RSD of 23% or less for the 50pg/μL point except for 1,2,3,4,6,7,8 HpCDD (%RSD = 31%). The average % RSD for all congener groups was 17.1%. This data shows the variation that can be expected in a continuing calibration point as opposed to the variation of RRFs from different concentration points in the same curve.

The data generated can also be used to determine instrument stability. For this analysis the mean relative response factor that was obtained for a specific compound between the initial analysis and 8 hours, 24 hours and 92 hours later was compared. As stated previously the instrument was not retuned or recalibrated during this period and thus any changes can be attributed to instrument variability. Overall, good agreement (less than 20% RSD) was obtained between the mean relative response factors over 92 hours. Higher relative standard deviations (40-80%) were observed for 1,2,3,4,6,7,8-HpCDD, OCDD and OCDF. A reproducibility of about 20% for this period of time was observed for the other HpCDD isomers and HpCDF and thus we cannot explain this occurrence solely on differences among congeners.

Table 3.1 Relative Response Factors of the Analytes to the Internal Standards Obtained by the Six Separate Analyses of Standard Solutions

Analyte	Mean Relative Response Factor ± S.D. (%RSD)					
	t1	t2 (8hr)	t3 (24hr)	t4 (92hr)	t5 (3mo)	t6 (7mo)
2,3,7,8-TCDD	1.096±0.168 (15)	1.173±0.214 (18)	1.476±0.053 (4)	1.920±0.466 (24)	1.511±0.121 (8)	1.768±0.216 (12)
2,3,7,8-TCDF	1.415±0.136 (10)	1.650±0.154 (9)	2.013±0.092 (5)	1.805±0.573 (32)	1.996±0.213 (11)	1.970±0.202 (10)
1,2,3,7,8-PeCDD	1.340±0.117 (9)	1.334±0.096 (7)	1.738±0.168 (10)	1.795±0.305 (17)	1.744±0.133 (8)	1.657±0.234 (14)
1,2,3,7,8-PeCDF	1.637±0.210 (13)	1.581±0.186 (12)	1.122±0.054 (5)	0.986±0.306 (31)	1.491±0.160 (11)	1.272±0.109 (9)
2,3,4,7,8-PeCDF	1.594±0.123 (8)	1.570±0.069 (4)	1.224±0.071 (6)	0.962±0.272 (28)	1.487±0.127 (9)	1.416±0.189 (13)
1,2,3,4,7,8-HxCDD	0.985±0.191 (19)	1.082±0.199 (19)	1.215±0.088 (7)	1.411±0.115 (8)	1.278±0.109 (9)	1.310±0.099 (8)
1,2,3,6,7,8-HxCDD	1.011±0.106 (11)	1.129±0.163 (14)	1.244±0.127 (10)	1.442±0.125 (9)	1.231±0.095 (8)	1.310±0.099 (8)
1,2,3,7,8,9-HxCDD	1.061±0.113 (11)	1.149±0.163 (14)	1.339±0.108 (8)	1.436±0.123 (9)	1.278±0.109 (9)	1.322±0.058 (5)
1,2,3,4,7,8-HxCDF	1.209±0.180 (15)	1.286±0.243 (19)	0.807±0.111 (14)	0.952±0.049 (5)	1.068±0.072 (7)	1.040±0.067 (6)
1,2,3,6,7,8-HxCDF	1.312±0.042 (3)	1.291±0.174 (14)	1.326±0.231 (17)	0.834±0.088 (11)	0.948±0.099 (10)	1.068±0.072 (7)
2,3,4,6,7,8-HxCDF	1.122±0.124 (11)	1.306±0.298 (23)	0.759±0.086 (11)	0.905±0.217 (24)	0.913±0.071 (8)	0.940±0.048 (5)
1,2,3,7,8,9-HxCDF	0.950±0.088 (9)	1.111±0.435 (39)	0.742±0.143 (19)	0.823±0.122 (15)	0.879±0.052 (6)	0.823±0.033 (4)
1,2,3,4,6,7,8-HpCDD	1.311±0.224 (17)	1.177±0.122 (10)	2.607±0.179 (7)	3.598±0.298 (8)	2.255±0.102 (5)	2.150±0.096 (5)
1,2,3,4,6,7,8-HpCDF	1.159±0.142 (12)	1.125±0.134 (12)	1.530±0.338 (22)	1.550±0.215 (14)	1.561±0.163 (10)	1.391±0.231 (17)
1,2,3,4,7,8,9-HpCDF	0.929±0.099 (11)	0.817±0.133 (16)	1.356±0.077 (6)	1.586±0.089 (6)	1.307±0.163 (13)	1.091±0.076 (7)
OCDD	0.644±0.226 (35)	0.600±0.111 (19)	0.588±0.054 (9)	0.201±0.082 (41)	0.866±0.084 (10)	0.747±0.088 (12)
OCDF	0.506±0.098 (19)	0.424±0.021 (5)	0.269±0.025 (9)	0.201±0.082 (41)	0.402±0.035 (9)	0.382±0.035 (9)

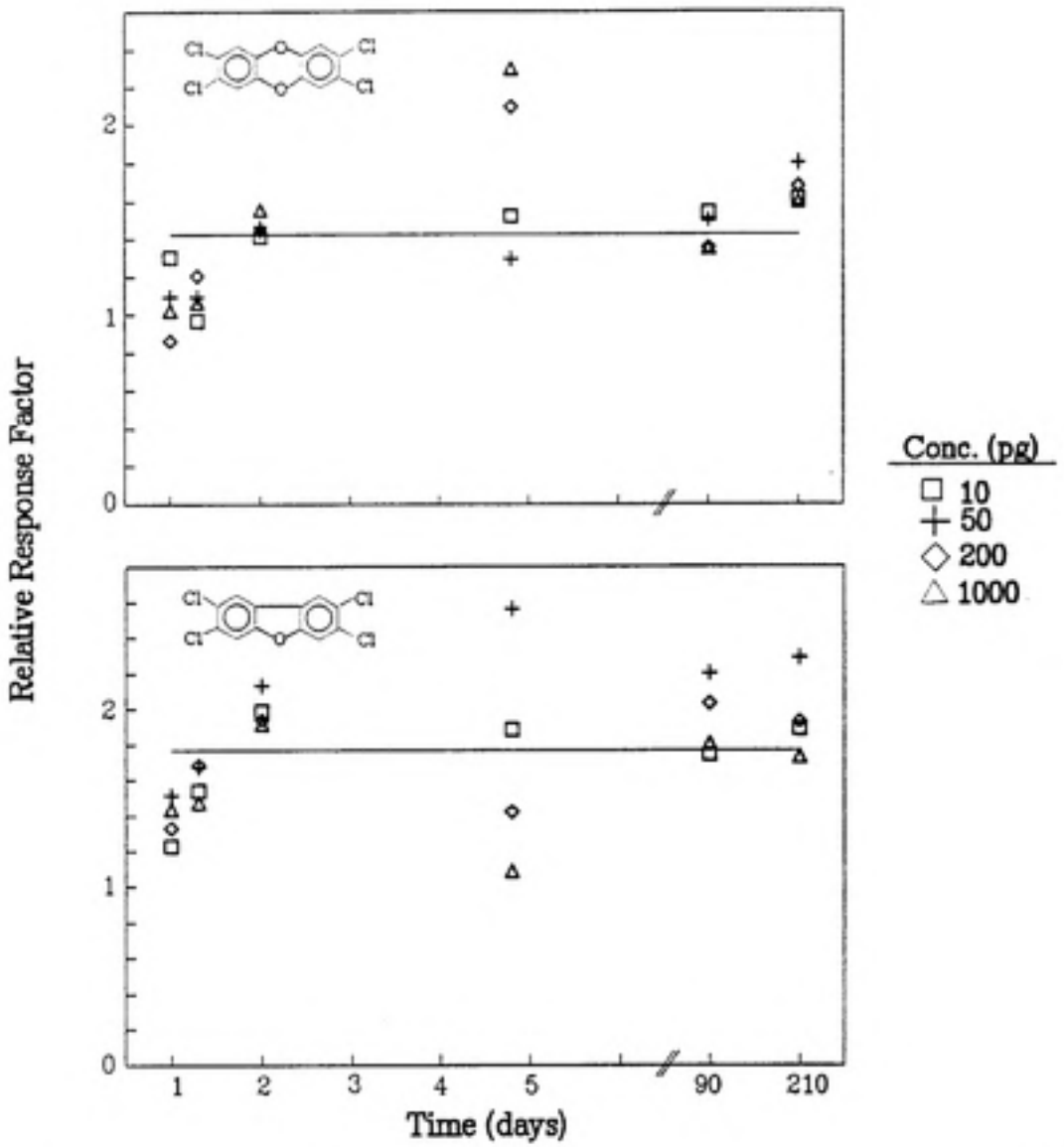

Figure 3.36A. The reproducibility and variability of the relative response factors (RRFs) from MS/MS analyses of calibration standards (2,3,7,8-TCDD and 2,3,7,8 TCDF). The variation in the RRFs increased over 4 days after instrument tuning on the first day. Calibration curves made by following the tuning protocol three and seven months later produced RRFs that demonstrated a return to the stability achieved in the first three curves. The line plotted is the mean value of all the RRFs.

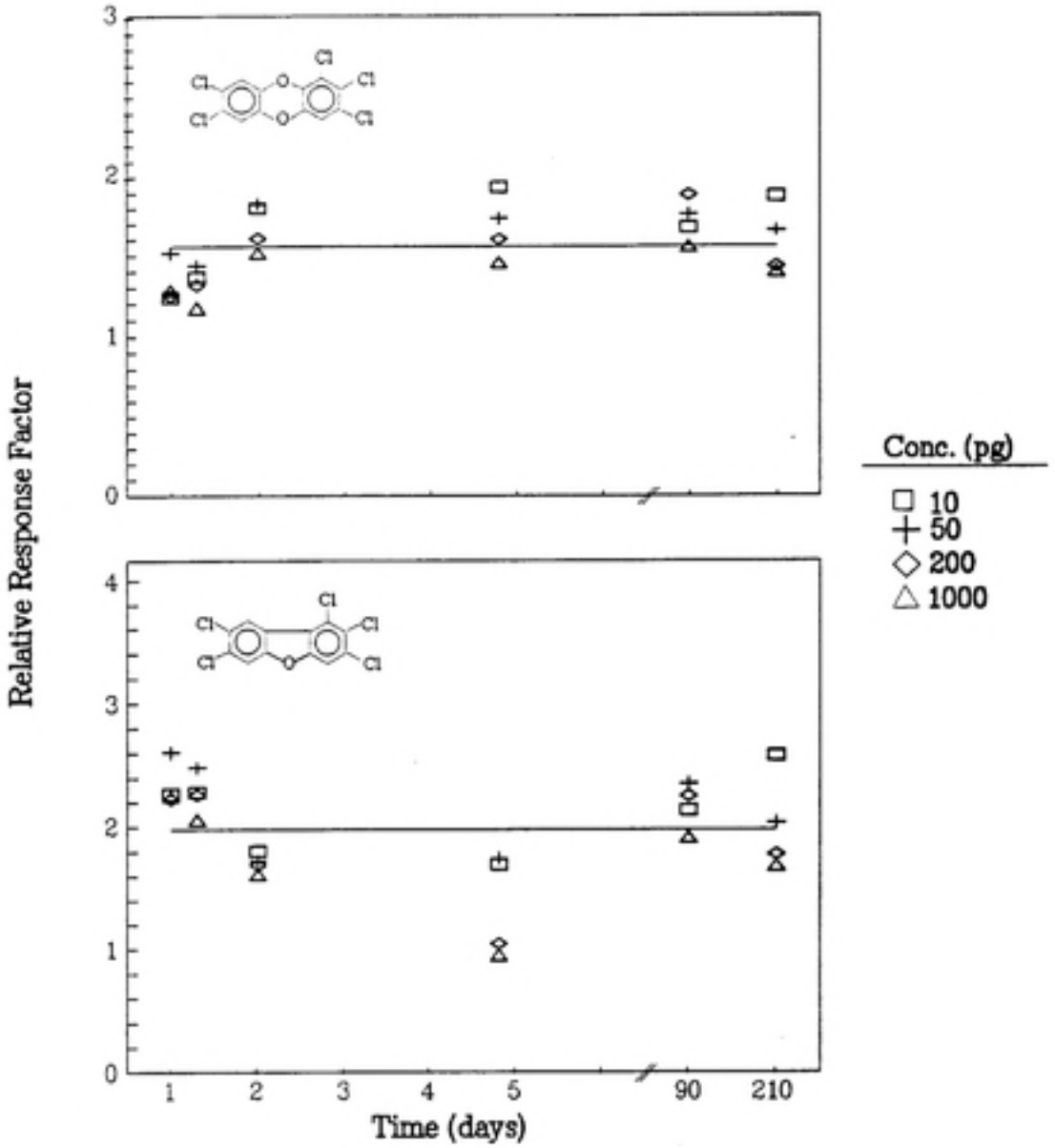

Figure 3.36B. The reproducibility of the relative response factors from MS/MS analyses of calibration standards (1,2,3,7,8-PeCDD and 1,2,3,7,8-PeCDF).

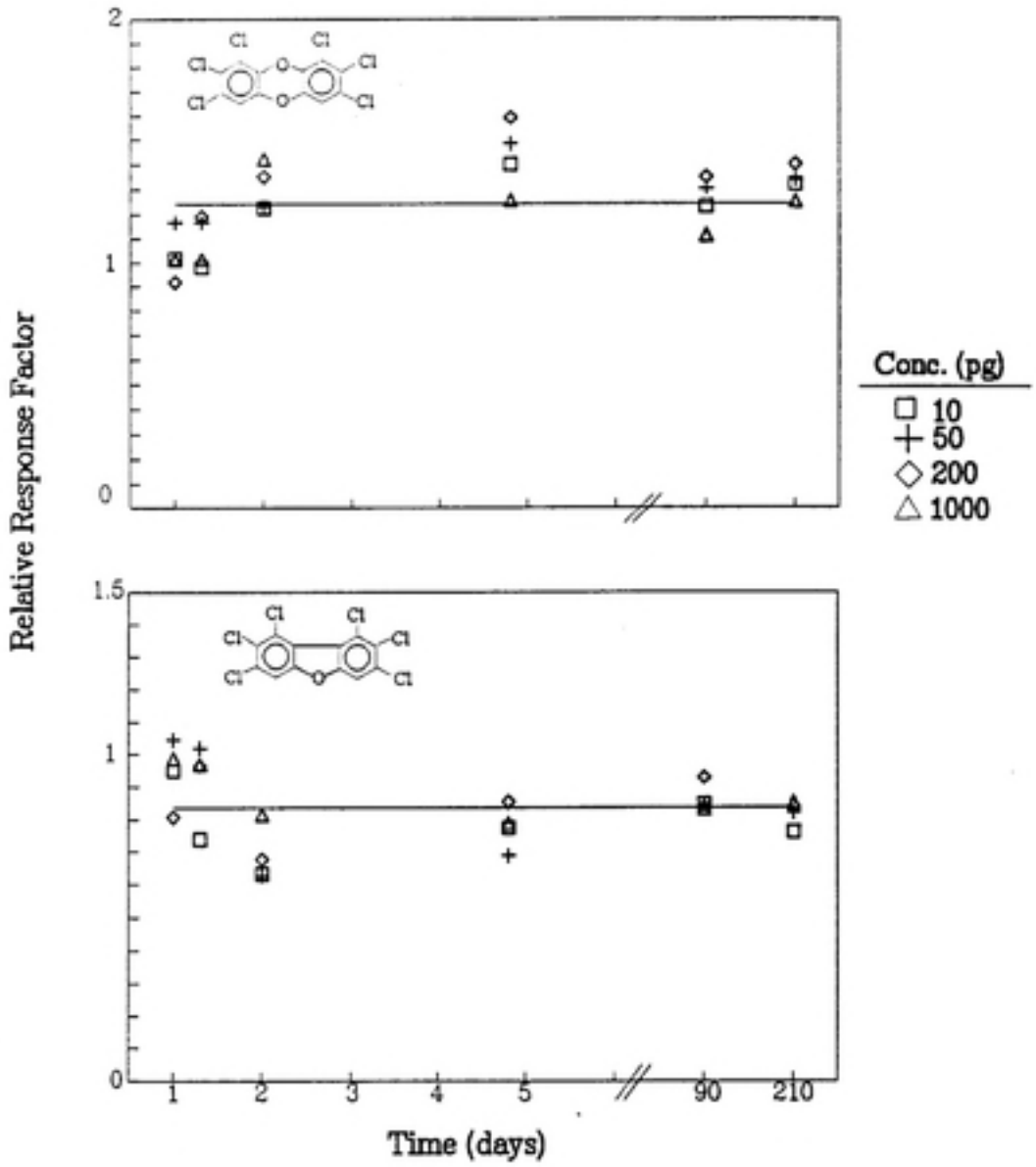

Figure 3.36C. The reproducibility of the relative response factors from MS/MS analyses of calibration standards (1,2,3,7,8,9-HxCDD and 1,2,3,7,8,9-HxCDF).

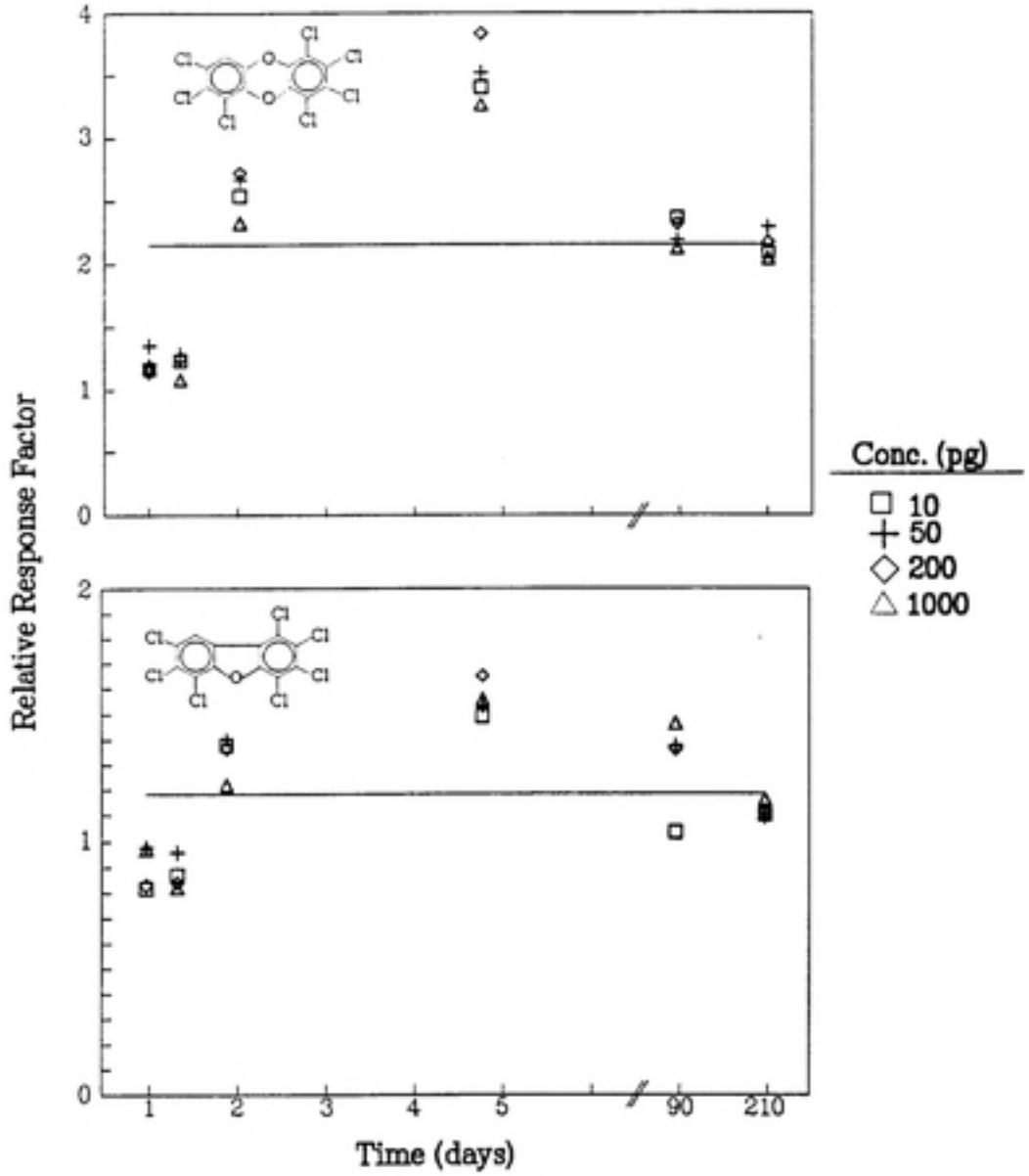

Figure 3.36D. The reproducibility of the relative response factors from MS/MS analyses of calibration standards (1,2,3,4,6,7,8-HpCDD and 1,2,3,4,6,7,8-HpCDF).

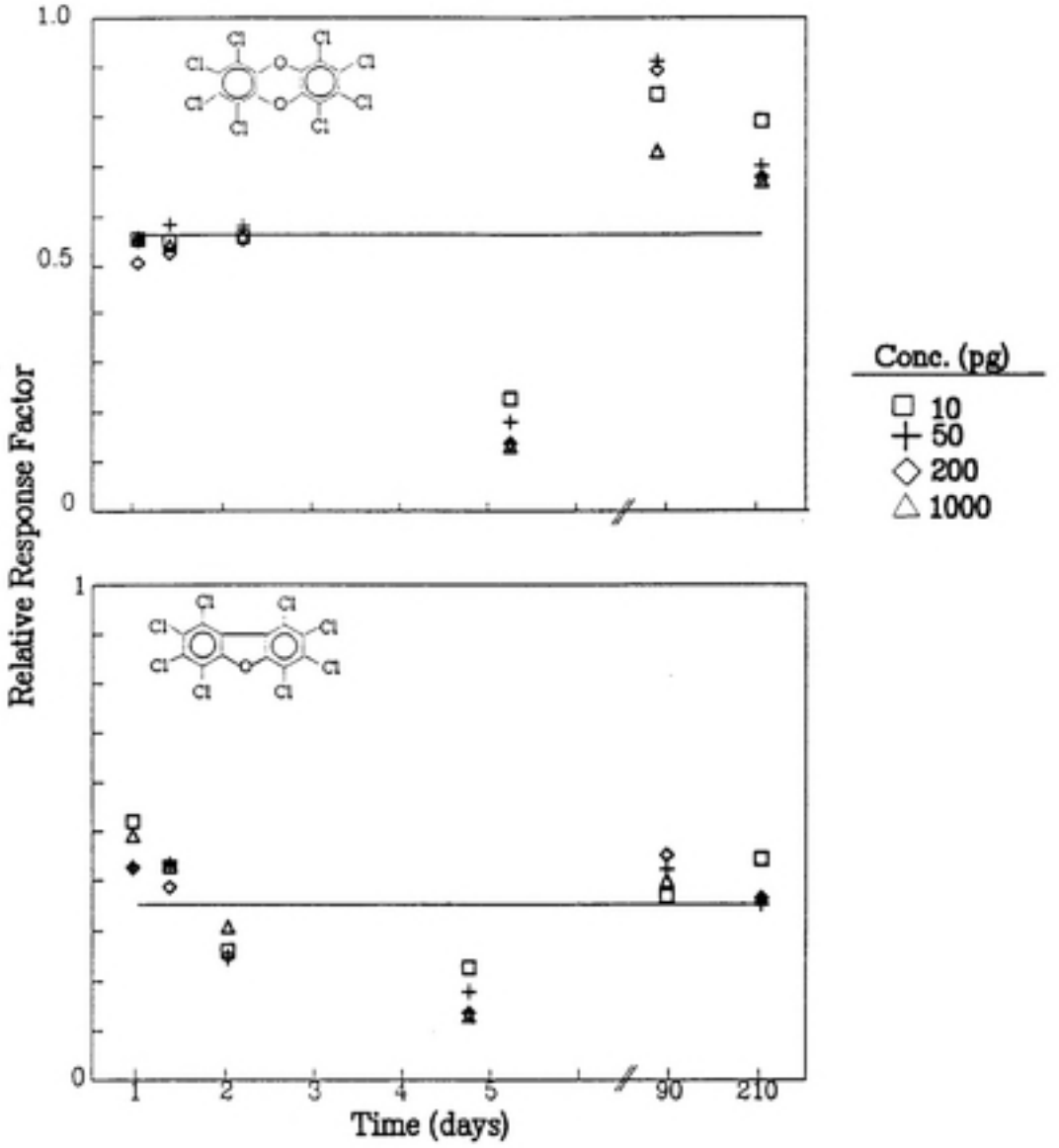

Figure 3.36E. The reproducibility of the relative response factors from MS/MS analyses of calibration standards (OCDD and OCDF).

Table 3.2 Variation in the Relative Response Factors from a 50 pg/μL Standard over a 7 month period

Analyte	Mean RRF[1] (S.D.)	%RSD
2,3,7,8-TCDD	1.392 (0.301)	21.7
2,3,7,8-TCDF	1.964 (0.345)	17.6
1,2,3,7,8-PeCDD	1.643 (0.164)	10.0
1,2,3,7,8-PeCDF	1.490 (0.238)	16.0
2,3,4,7,8-PeCDF	1.504 (0.209)	13.9
1,2,3,6,7,8-HxCDD	1.189 (0.109)	9.0
1,2,3,4,7,8-HxCDD	1.193 (0.128)	10.8
1,2,3,7,8,9-HxCDD	1.242 (0.081)	6.5
1,2,3,6,7,8-HxCDF	1.189 (0.226)	19.0
1,2,3,4,7,8-HxCDF	1.084 (0.214)	19.8
2,3,4,6,7,8-HxCDF	1.024 (0.233)	22.8
1,2,3,7,8,9-HxCDF	0.871 (0.169)	19.5
1,2,3,4,6,7,8-HpCDD	1.958 (0.607)	31.0
1,2,3,4,6,7,8-HpCDF	1.381 (0.167)	12.1
1,23,4,7,8,9-HpCDF	1.160 (0.213)	18.4
OCDD	0.667 (0.148)	22.3
OCDF	0.377 (0.078)	20.7

[1](n=5) The data from the 0hr, 8hr, 24hr, 3 mo and 7mo calibration curves were used.

All tuning and calibration curve work was performed under the premise that, if the instrument were in good working order, reproducible results could be obtained. The amount of time taken to conduct the study bore out this idea. As operator familiarity with the instrument increased, the time to tune the instrument and get acceptable RRFs from a continuing point decreased. Invariably, wide variation (30% difference or more) in an RRF could be traced to a tuning problem or instrument malfunction. Thus, a well-maintained MS/MS instrument tuned by a knowledgeable operator should provide a stable, linear response from calibration standards in the 2.5 to 1000 pg/μL range.

Isotope Ratio Measurements: The percent difference between the theoretical and measured isotope ratios for the standard compounds was calculated for each point of the calibration curve for all 17 PCDD/F congeners. The average percent difference of the five points within a calibration curve was calculated, and these means were averaged to get an overall percent difference value for each isomer for the 6 curves that were produced. The overall percent difference between theoretical and measured isotope rations ranged from 5% to 24%, the highest percentage difference being OCDF. The next highest difference was 19% (1,2,3,4,6,7,8 HpCDF). All other congeners had overall percent difference values of 15% or less (see Table 3.3). These data demonstrate that acceptable differences between the theoretical and measured isotope ratios can be defined and incorporated into identification criteria for identifying the analytes of interest when performing MS/MS analyses. The % difference limits between the measured and theoretical isotope ratio in HRMS Method 8290 are $\pm$ 15% for all congeners. The recommended limits for MS/MS are slightly higher: 20% for tetra, 25% for penta

through hepta, and up to 30% difference for OCDD/F.

Table 3.3 % Relative Difference Between Measured and Theoretical Isotope Ratios.

Analyte	Mean % Relative Difference ± S.D. Between Measured and Theoretical Isotope Ratios					
	t_1	t_2 (8 hr.)	t_3 (24 hr.)	t_4 (92 hr.)	t_5 (3 mo.)	t_6 (7 mo.)
2,3,7,8 TCDD	7.4±8.4	5.8±2.9	6.3±5.6	6.9±6.3	2.1±1.7	4.1±3.1
^{13}C-2,3,7,8 TCDD	6.8±3.3	9.9±3.0	25.9±7.3	21.1±2.5	8.6±4.6	4.8±4.1
2,3,7,8 TCDF	12.4±7.9	13.5±12.8	15.8±14.4	5.3±8.2	4.0±7.2	5.9±5.7
^{13}C-2,3,7,8 TCDF	11.5±4.8	6.4±2.5	22.7±7.8	21.0±12.2	5.3±3.4	4.3±4.7
2,3,7,8 PeCDD	5.3±5.9	5.3±5.3	2.2±2.0	7.0±5.3	4.1±1.5	5.2±3.0
1,2,3,7,8 PeCDF	15.2±8.3	10.4±2.9	12.8±5.6	10.2±12.1	10.3±5.3	14.6±4.9
^{13}C 1,2,3,7,8 PeCDF	8.3±6.4	10.2±5.2	20.3±8.0	18.0±4.7	3.9±5.0	7.5±7.3
2,3,4,7,8 PeCDF	11.0±6.6	14.0±3.0	19.6±14.5	11.2±8.2	5.6±1.9	7.6±0.8
1,2,3,4,7,8 HxCDD	10.1±5.5	21.5±9.2	7.9±2.9	16.2±18.4	16.5±4.3	21.4±7.7
1,2,3,6,7,8 HxCDD	14.5±12.6	22.1±9.6	7.4±4.1	8.8±4.7	19.6±12.3	21.4±7.7
1,2,3,7,8,9 HxCDD	12.8±6.5	25.1±13.3	5.8±3.3	15.4±14.8	16.5±4.3	15.4±11.0
^{13}C 1,2,3,7,8,9 HxCDD	16.3±4.2	19.8±7.2	27.6±8.7	21.0±7.8	21.1±7.8	17.9±6.7

Table 3.3. % Relative Difference Between Measured and Theoretical Isotope Ratios (continued).

Analyte	Mean % Relative Difference ± S.D. Between Measured and Theoretical Isotope Ratios					
	t_1	t_2 (8 hr.)	t_3 (24 hr.)	t_4 (92 hr.)	t_5 (3 mth.)	t_6 (7 mth.)
2,3,4,6,7,8 HxCDF	11.0±5.6	11.6±4.0	17.7±3.5	16.8±16.6	7.4±7.9	11.6±8.7
1,2,3,7,8,9 HxCDF	13.9±4.3	17.8±5.1	13.2±5.4	18.3±6.7	8.8±6.8	11.6±8.1
1,2,3,6,7,8 HxCDF	12.5±4.7	16.8±3.1	11.8±1.9	11.0±11.7	6.7±3.9	7.9±5.5
1,2,3,4,7,8 HxCDF	11.2±3.9	14.3±4.8	16.5±7.1	6.9±5.1	6.7±3.9	7.9±5.5
1,2,3,4,6,7,8 HpCDD	4.5±2.3	11.3±6.3	5.5±2.4	13.1±9.1	8.7±4.4	4.7±3.1
1,2,3,4,6,7,8 HpCDF	25.9±10.0	29.3±4.5	18.3±5.6	18.6±14.5	10.7±5.4	10.5±5.7
^{13}C-1,2,3,6,7,8 HpCDF	10.5±4.6	13.5±6.7	19.2±4.5	18.9±10.5	8.0±4.0	10.4±9.6
1,2,3,4,7,8,9 HpCDF	16.3±6.6	22.4±3.9	14.9±9.7	11.5±6.6	8.7±3.4	14.0±8.1
OCDD	12.4±22.2	1.8±1.9	12.1±6.9	31.2±12.9	16.9±23.0	9.8±4.9
^{13}C-OCDD	13.1±5.9	13.0±7.0	19.9±3.7	33.4±13.1	19.3±4.8	5.6±1.9
OCDF	28.4±12.0	24.9±13.1	39.3±12.5	31.2±12.9	12.3±2.8	8.4±3.1

Quantitation of Sample Extracts:

Comparison of HRMS and MS/MS

The four extracts (three replicates and one a matrix spike) from the fly ash samples were first analyzed and quantified by mass spectrometry/mass spectrometry. Analysis of this extract represents the case in which compounds that interfere with the quantitation of PCDD/Fs are absent and thus may be used as a direct comparison of HRMS and MS/MS (Table 3.4). A statistical analysis was performed on the resulting data to determine whether the mean concentration values from each method were significantly different. The statistical methods used included individual t-tests on each congener group (e.g., total TCDDs) and for specific isomers from each congener group.

The t-tests were used to determine whether the mean values of the congener groups were significantly different at a 95% confidence level. The use of multiple paired t-tests increases the Type 1 error rate (finding the means significantly different when they are actually not). In addition, paired t-tests could only be applied to values from the same congener group and precluded treating the data from one method as a whole. So, to control for the possible inflated error rate of multiple t-tests and to account for the repeated measure nature of the date, we also performed a univariate approach to repeated measures analysis. Such an approach increases the number of degrees of freedom of the test and, thus, affords greater power to the test statistic by using all observations at once, rather than the congener group by congener group method of the t-tests. The univariate approach allowed for the treatment of the data as a single group and permitted a direct

Table 3.4A

Precision of the MS/MS Method Quantitation of Fly Ash Samples

CONGENER	CONCENTRATION OF REPLICATE (pg/mg) 1	2	3	MEAN (S.D.)	%RSD
Total TCDD	115.2	213.8	172.6	167.2 ± 49.5	30
2378-TCDD	10.2	16.4	22.3	16.3 ± 6.0	37
Total TCDF	367.8	329.0	421.7	372.8 ± 46.5	12
2378-TCDF	43.3	37.8	56.8	34.2 ± 9.0	27
Total PeCDD	265.2	219.4	251.3	245.3 ± 23.5	10
12378-PeCDD	17.3	10.7	16.3	14.8 ± 3.5	24
Total PeCDF	214.5	204.2	336.7	251.8 ± 73.7	29
12378-PeCDF	13.8	6.6	7.8	9.4 ± 3.8	41
Total HxCDD	405.5	385.9	484.0	425.1 ± 51.9	12
123789-HxCDD	12.9	16.8	16.8	15.5 ± 2.2	15
Total HxCDF	188.9	175.0	246.3	203.4 ± 37.8	19
234678-HxCDF	17.7	20.6	28.9	22.4 ± 5.8	26
Total HpCDD	327.4	300.9	382.6	337.0 ± 41.7	12
1234678-HpCDD	167.8	140.8	195.1	167.9 ± 27.1	16
Total HpCDF	88.4	84.6	116.4	96.5 ± 17.4	18
1234678-HpCDF	57.0	55.5	89.9	67.4 ± 19.4	29
OCDD	272.4	197.6	252.6	240.9 ± 38.8	16
OCDF	37.0	24.0	41.5	34.2 ± 9.1	27

Table 3.4B Precision of the Fly Ash Extract Quantitation
By High Resolution Mass Spectrometry

CONGENER	CONCENTRATION OF REPLICATE (pg/mg) 1	2	3	MEAN (S.D.)	%RSD
Total TCDD		161.6	183.7	172.7 ± 15.6	9
2378-TCDD		8.5	17.3	12.9 ± 6.2	48
Total TCDF		315.8	352.6	334.2 ± 26.0	8
2378-TCDF		37.6	84.2	60.9 ± 32.9	54
Total PeCDD		243.8	267.5	255.7 ± 16.8	7
12378-PeCDD		10.2	18.0	14.1 ± 5.5	39
Total PeCDF		212.3	319.5	265.9 ± 75.8	29
12378-PeCDF		13.8	12.0	12.9 ± 1.3	10
Total HxCDD		410.0	520.1	465.1 ± 77.9	17
123789-HxCDD		28.3	28.4	28.4 ± 0.1	1
Total HxCDF		235.8	276.3	256.1 ± 28.6	11
234678-HxCDF		19.4	25.6	22.5 ± 4.4	19
Total HpCDD		320.2	365.9	343.1 ± 32.3	9
1234678-HpCDD		164.2	184.6	174.4 ± 14.4	8
Total HpCDF		102.3	123.5	112.9 ± 14.9	13
1234678-HpCDF		72.2	96.4	84.3 ± 17.1	20
OCDD		248.6	332.0	290.3 ± 58.9	20
OCDF		34.8	34.8	34.8 ± 0.1	1

comparison of the complete set of quantitation values obtained from the two methods.

T-tests on the mean concentration values for total PCDDs and PCDFs showed no significant difference at the .05 level. The t-tests performed on the individual isomers yielded the same result for all isomers except for the 123789 HxCDD isomer, whose t-test for the two methods showed a significant difference between the means (p-value of 0.005 with pooled variance). The test of the methods by univariate approach to repeated measures analysis showed a non-significant difference at the 0.05 level ($F = 0.30$ with 1 and 3 degrees freedom and a p-value = 0.6235). This statistical method also allowed for a test of difference between the two methods by congener group. Again, the interaction between method and congener was non-significant ($F = 0.91$ with 9 and 27 degrees of freedom and a p-value =0.5295). The univariate approach was not applied to the specific isomer values because they were considered a subset of the total congener values, and thus, were treated in the first analysis. Both the t-tests and the more powerful univariate approach to repeated measures analysis show that the mean quantitation values of tetra through octa PCDDD/Fs in the samples determined by both high resolution mass spectrometry and tandem mass spectrometry were not significantly different at the 95% confidence level.

The sample extract containing the matrix spiking solution of a known quantity of unlabeled PCDD/Fs was analyzed and quantitated in the same way as the other samples by HRMS and MS/MS. The purpose of quantifying the matrix spike samples was to determine the accuracy of the MS/MS method and compare its performance to the accuracy of HRMS. The results in Table 3.5 show that both methods are comparable

in their differences from the expected concentration results. The average percent difference from the expected concentration value was 19% for HRMS and 11% for MS/MS. Excluding the OCDF and OCDD, which were different by 41% and 35% for HRMS and 20% and 25% for MS/MS, the average % difference between the quantitated concentration value and the expected was 14% for HRMS and 8% for MS/MS. The accuracy criteria for EPA method 8290 calls for the measured value of the matrix spike to be 70% to 130% of target values. In our example, all the isomers as quantified by MS/MS fell with the limits. We have demonstrated in this case that MS/MS analysis is as accurate as HRMS and therefore would recommend the same accuracy limits as the HRMS 8290 method.

The Hudson River Sediment Samples

The three extracts and the laboratory blank from the sediment samples were also analyzed by both HRMS and MS/MS. The HRMS analysis in all three samples showed the presences of materials that interfered with the quantitation of PCDD/Fs. Specifically, the materials affected quantitation of the PeCDDs. The isotope ratio of the PeCDD $(M+2)^+$ and $(M+4)^+$ ions monitored fell outside the acceptable deviation limits (20%). The ratios were all 35-40% below the theoretical value of 1.55. In addition, the chromatogram of the PFK lock mass check exhibited as much as a 50% fluctuation in the PeCDD window, which can be interpreted as source de-tuning (Figure 3.4). The chromatograms in figure 3.40 show the high signals detected in the $(M+4)^+$ PeCDD ion, the PFK lock mass check deflection, and the trace of the $(M+2)^+$ ion as well. Note that the greatest deflection of the lock mass check trace occurs well after the large signals

Table 3.5 **A Comparison of the analyis of PCDDs and PCDFs in a Matrix Spike by using High Resolution Mass Spectrometry and Mass Spectrometry/Mass Spectrometry**

Analyte	Expected Concentration (pg/g)	Measured Concentration (% Relative Difference) Method	
		HRMS	MSMS
2,3,7,8-TCDD	827	1074 (30)	953 (15)
2,3,7,8-TCDF	827	959 (16)	803 (-3)
1,2,3,7,8-PeCDD	827	930 (13)	767 (-7)
1,2,3,7,8-PeCDF	827	951 (15)	774 (-6)
1,2,3,7,8,9-HxCDD	827	852 (4)	790 (5)
2,3,4,6,7,8-HxCDF	827	946 (14)	946 (14)
1,2,3,4,6,7,8-HpCDD	827	862 (4)	893 (7)
OCDD	827	1117 (35)	1069 (25)
OCDF	892	1258 (41)	1069 (20)

appear. These particular chromatograms show the presence of both mass interferants and unspecified "matrix effects" within the same sample. An explanation for the observation of possible matrix effects might come from the total ion chromatogram (TIC) of the full scan analysis of the sediment extract (figure 3.55). The TIC indicates that, even after a clean-up procedure, a large amount of unidentified materials remain at concentrations estimated to be 1000 times higher than the PCDD/Fs. The results of the MS/MS analysis show the effective removal of the mass interferants and allowed quantitation of the PeCDDs, including 1,2,3,7,8 PeCDD. (figure 3.50). Analytical results by both methods are listed in Table 3.6. The presence of a mass interferant in the $(M+4)^+$ PeCDD channel prompted an analysis for PeCDD by HRMS using the M^+ and $(M+2)^+$ channels (instead of the $(M+2)^+$ and $(M+4)^+$). While no large mass interferant was evident in these two channels, the majority of the detected peaks were outside the acceptable isotope ratio limits for HRMS. In this case, MS/MS was the only method that effectively removed the interferants.

Identification of Mass Interferants

As mentioned above, the HRMS analysis of the sediment extracts exhibited evidence of mass interference as well as non-specific interference (lock mass check deflection). The chromatograms of the HRMS analysis of PeCDD showed some high intensity peaks in the $(M+4)^+$ PeCDD (m/z 357.8516) channel that did not have corresponding signals in the $(M+2)^+$ channel (m/z 355.8546). A low resolution (1000) full scan mass (from m/z 500 to m/z 50) spectrum of the sample of the sample extract showed the presence of characteristic chlorine clusters, including a six chlorine cluster at nominal m/z 358. A

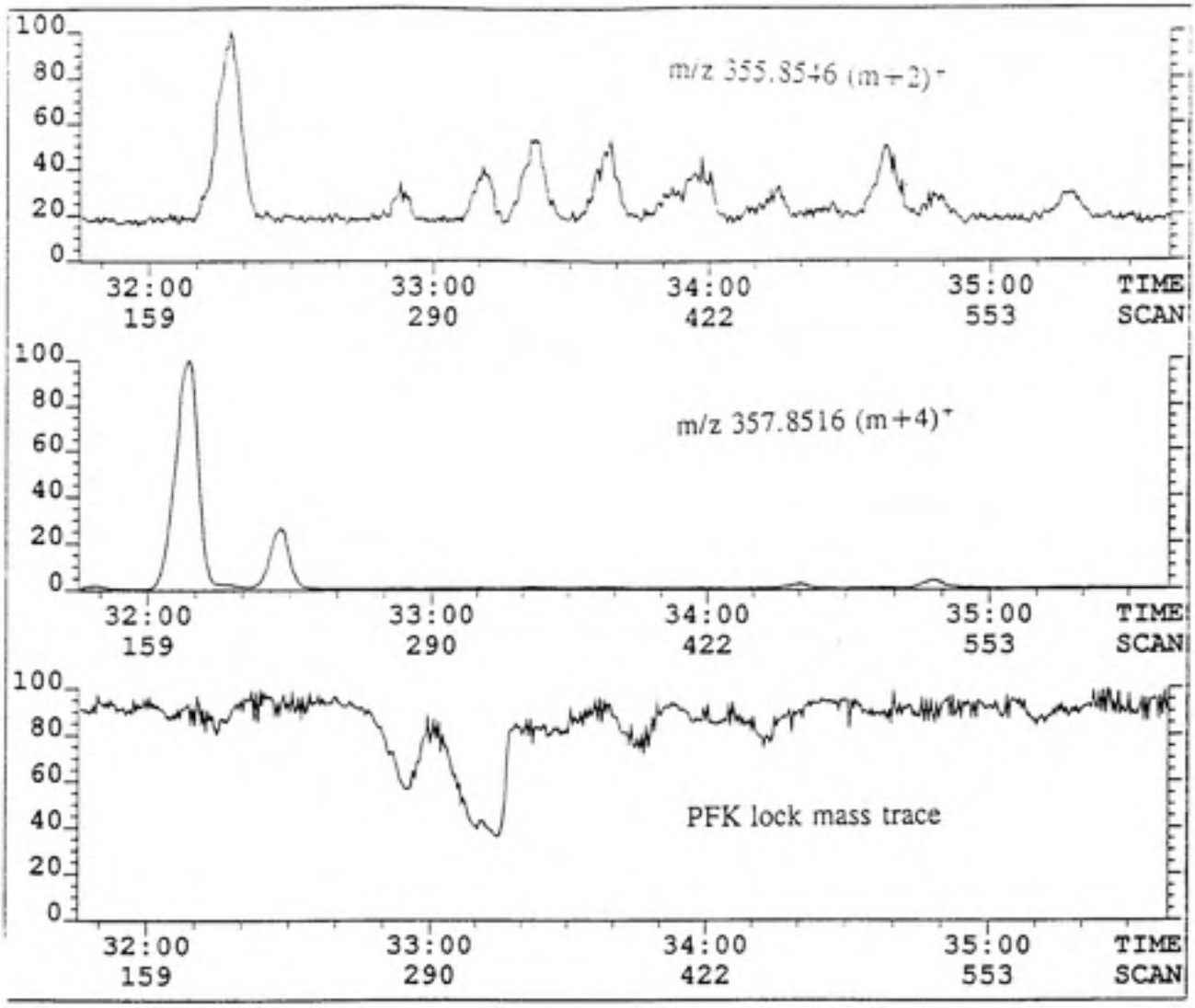

Figure 3.40. Selected Ion Monitoring Chromatograms from HRMS PeCDD analysis of Hudson River sediment extract showing the presence of an interfering signal in the $(M+4)^+$ channel (m/z 357.8516). Also shown is the trace of the PFK lock mass check exhibiting strong deflection possibly due to the "matrix effect" from other materials present in the extract.

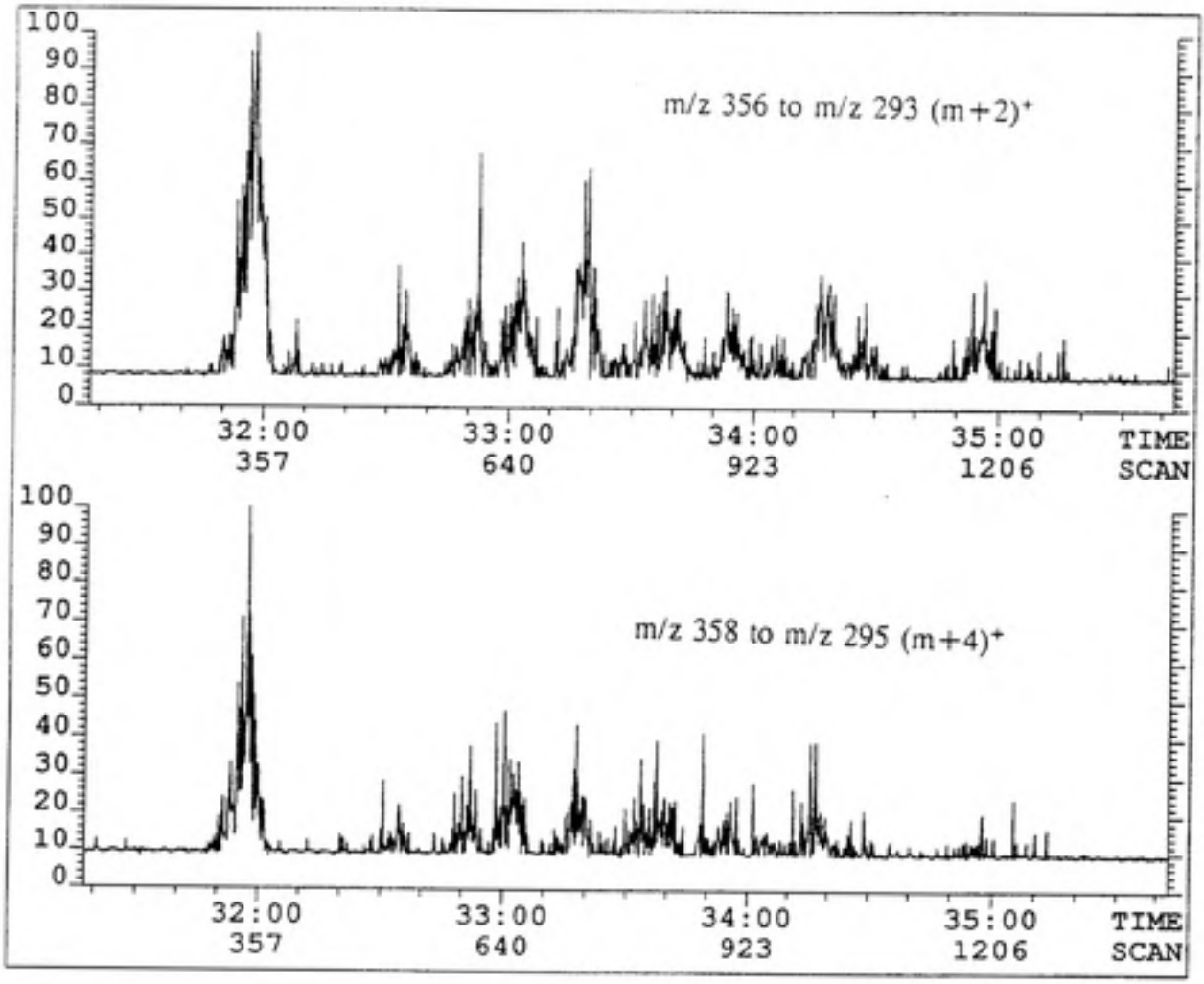

Figure 3.50. Selected Reaction Monitoring chromatograms from MS/MS analysis of Hudson River sediment extract for PeCDDs. The interfering peak that was present in the HRMS analysis has been removed.

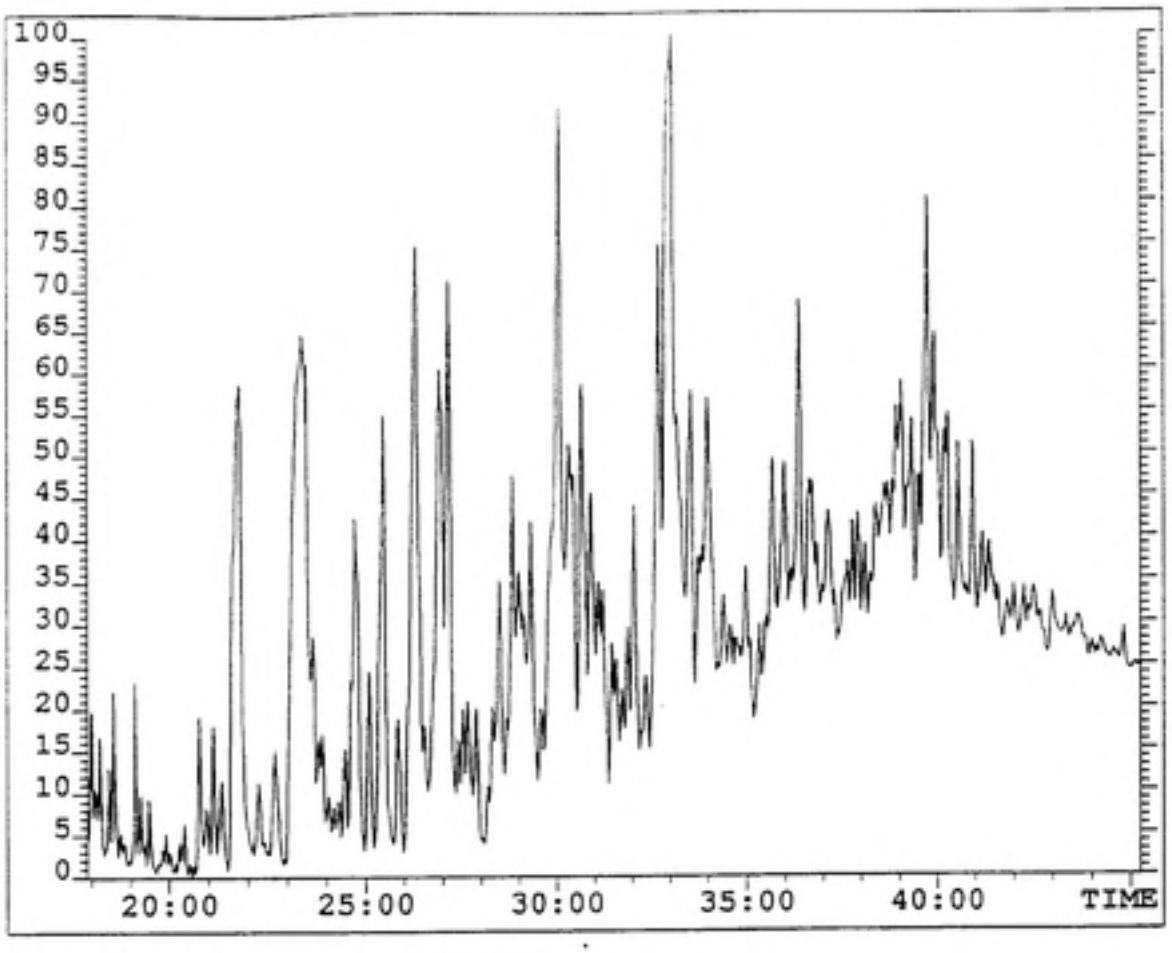

Figure 3.55. Total Ion Chromatogram from full scan analysis (m/z 500 to m/z 50) of Hudson River sediment extract showing the complexity of the extract even after sample clean-up procedure.

Table 3.6 A Comparison of High Resolution Mass Spectrometry and Mass Spectrometry/ Mass Spectrometry for the Analysis of PCDDs and PCDFs in a River Sediment Extract

Mean ± S.D. (n=2)
(% Relative Standard Deviation)

Concentration (ng/g)

Analyte	HRMS	MS/MS
Total TCDDs	0.14 ± 0.003 (20)	0.054 ± 0.009 (17)
2,3,7,8-TCDD	0.14 ± 0.003 (20)	0.054 ± 0.009 (17)
Total TCDFs	4.798 ± 0.725 (15)	3.909 ± 0.737 (19)
2,3,7,8-TCDF	0.993 ± 0.266 (27)	0.745 ± 0.142 (19)
Total PeCDDs	---	0.079 ± 0.005 (6)
1,2,3,7,8-PeCDD	---	0.011 ± 0.001 (1)
Total PeCDFs	1.256 ± 0.275 (22)	0.566 ± 0.042 (7)
2,3,4,7,8-PeCDF	0.118 ± 0.045 (38)	0.058 ± 0.005 (9)
Total HxCDDs	0.320 ± 0.061 (19)	0.337 ± 0.083 (25)
1,2,3,7,8,9-HxCDD	0.032 ± 0.007 (22)	0.045 ± 0.014 (31)
Total HxCDFs	1.444 ± 0.197 (19)	1.274 ± 0.418 (33)
1,2,3,7,8,9-HxCDF	0.060 ± 0.007 (12)	0.040 ± 0.008 (21)
Total HpCDDs	1.111 ± 0.235 (21)	1.455 ± 0.064 (4)
1,2,3,4,6,7,8-HpCDD	0.420 ± 0.002 (1)	0.610 ± 0.028 (5)
Total HpCDFs	0.549 ± 0.098 (18)	0.495 ± 0.049 (10)
1,2,3,4,6,7,8-HpCDF	0.202 ± 0.111 (55)	0.270 ± 0.014 (5)
OCDD	3.559 ± 0.836 (23)	3.537 ± 1.051 (30)
OCDF	0.224 ± 0.048 (22)	0.224 ± 0.062 (28)

five chlorine and four chlorine cluster were observed at m/z 323 and m/z 290, respectively (see figure 3.65). This spectrum resembles the that of a hexachlorinated biphenyl (figure 3.65). Additional identification information was obtained by performing an HRMS selected ion monitoring experiment for Hexa PCBs on one of the sediment extract. Three ions (M^+, $(M+2)^+$, $(M+4)^+$) in the molecular ion isotope cluster were monitored, as well as two ions for both the (M - Cl) and (M - 2Cl) fragment ions. The chomatograms from the experiment are shown in Figure 3.70. The isotope ratios of the resulting signals were within $\pm$ 15% of the theoretical isotope ratios for six, five and four chlorine clusters. In addition, it was shown that the peaks in question do co-elute within the PeCDD window. These results, along with the fact that a resolving power of 50000 would be needed to separate the M^+ ion of a Hexa PCB (m/z 357.8444) from the $(M+4)^+$ ion of a PeCDD (m/z 357.8516), make plausible that at least some of the mass interferants observed in the PeCDD chromatograms are Hexa PCBs.

The analysis of the Hudson River sediment extracts is an excellent example of using multiple mass spectrometry techniques in a complimentary fashion to obtain information about the samples than would not be possible by using just one technique. Most of the PCDD/F congener groups were readily analyzed by HRMS. The MS/MS made possible quantitation of PeCDDs in the extracts. The low resolution full scan data and HRMS selected ion monitoring data gave the necessary information for identification of some interferants.

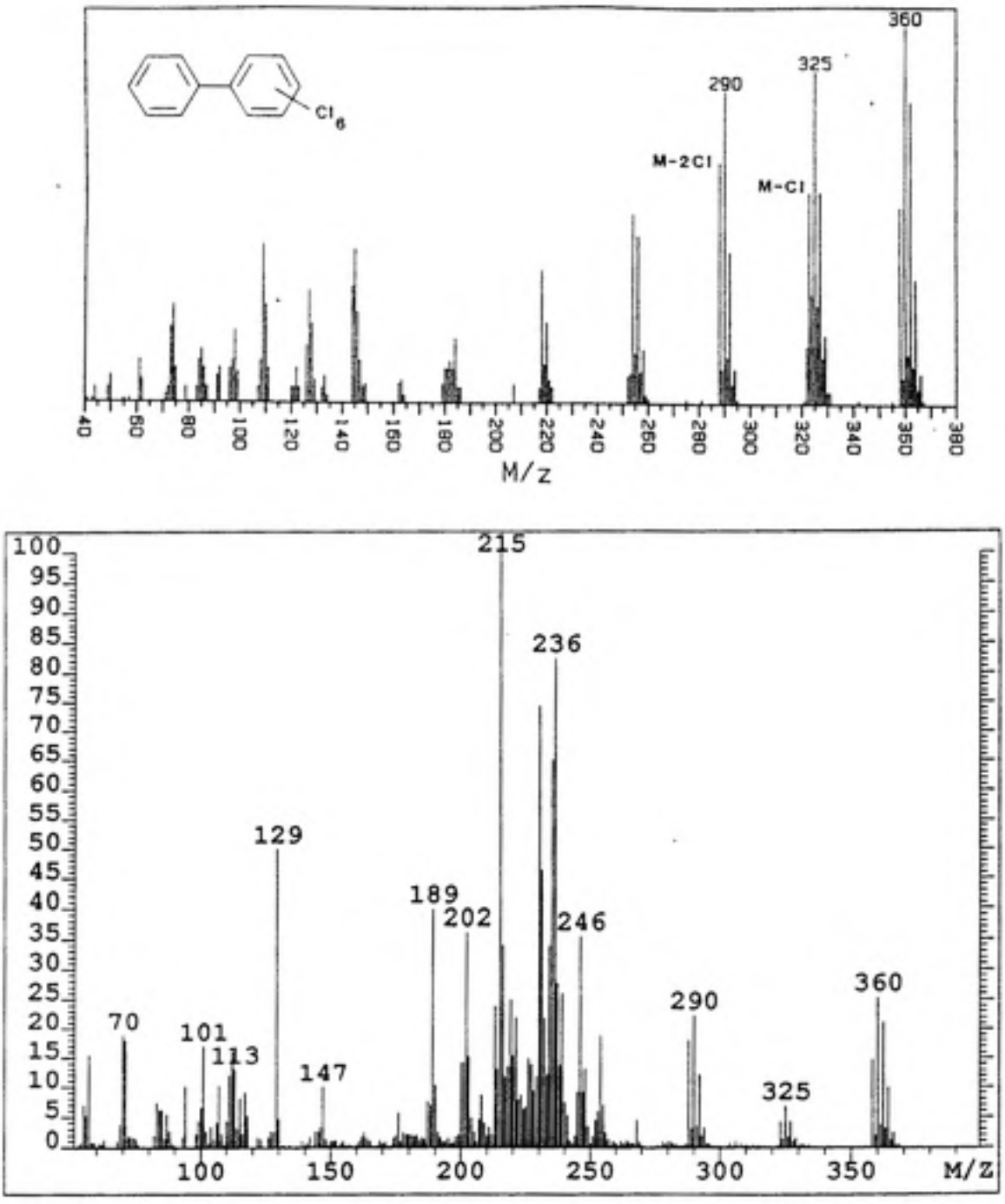

Figure 3.65. Reference mass spectrum of a Hexachlorinated biphenyl (top) for comparison to mass spectrum from the sediment extract.

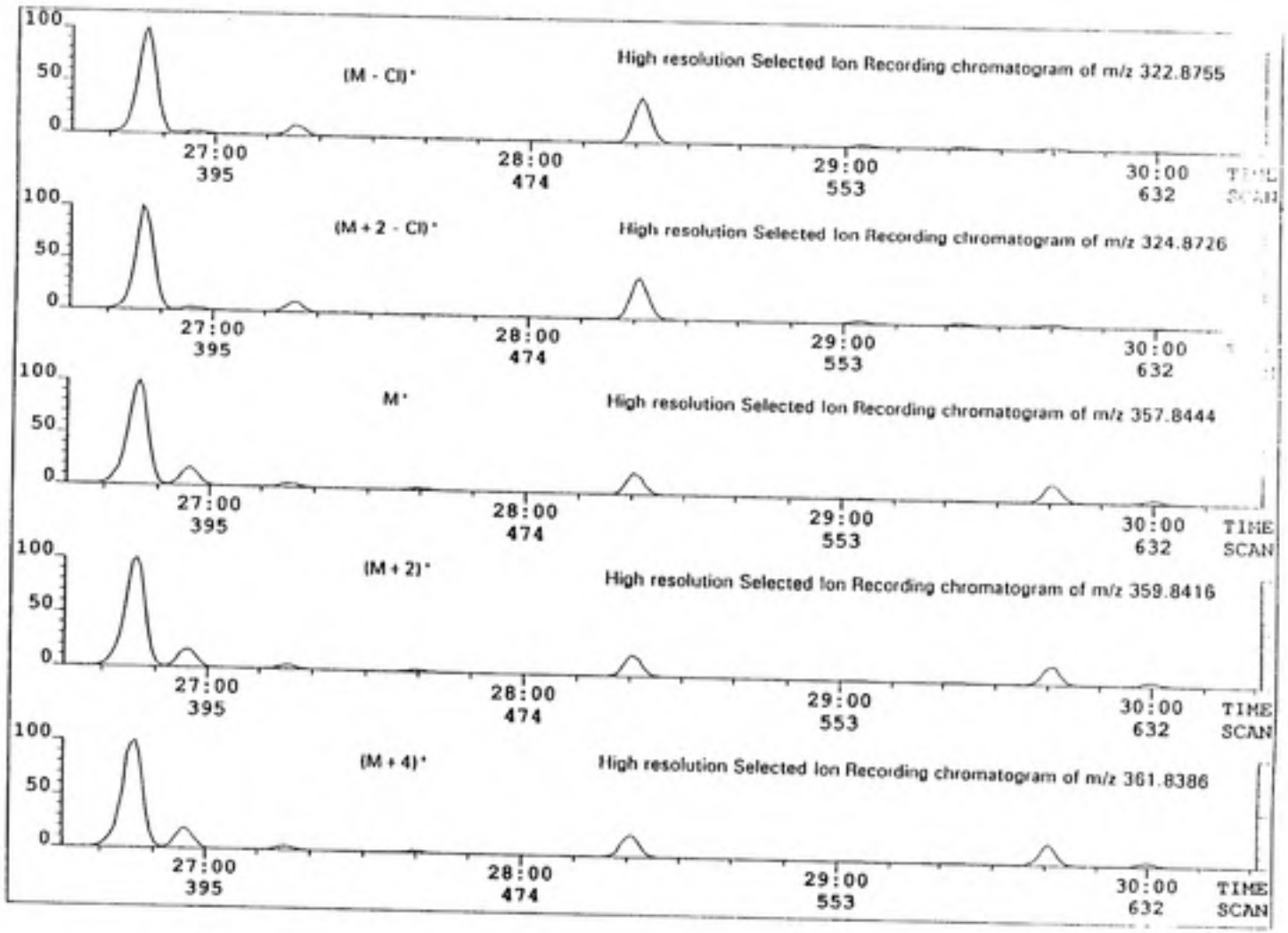

Figure 3.70. Selected ion recording chromatograms from HRMS analysis of Hudson River sediment extract for Hexa PCBs. The similarity of signal pattern in the molecular m/z channels (M, M+2, M+4) as well as in the M - Cl channels is the expected response for Hexa PCBs.

CONCLUSIONS

The study has demonstrated the stability of an MS/MS instrument for short periods (24 hours or less) and longer periods (months). The linearity of the response of the analyte to the internal standard over a concentration range of 2.5 to 1000 pg/μL was demonstrated repeatedly. The ability to generate relative response factors varying by less than 20-25 %RSD was shown. In addition, the study shows that, by following a tuning protocol, the same results for relative response factors can be reproduced months later. The difference between the measured isotope and theoretical value for tetra through octa PCDD/F congeners is 20-25% for tetra through hepta and about 30% for octa. The study defined the operational and tuning parameters necessary to conduct picogram level analysis of PCDD/Fs. The PCDD/Fs studied exhibited a range of collision energy optima (20-30 eV). The collision energy maxima for PCDDs and PCDFs are the same. PCDFs exhibited a broader maxima range than PCDDs, however. Follow up experiments investigating the cause of this difference led to the conclusion that PCDFs had the broader maxima because the M - COCl loss in PCDFs were not subject to competing fragmentation reactions (M-2COCl for example) as was the case observed with PCDDs. Isomeric differences in collision energy maxima were not observed.

When compared to the mean values of tetra through octa PCDD/Fs obtained by high resolution mass spectrometry, the mean quantitation values obtained by

MS/MS from fly ash samples were not significantly different (at a 95% confidence level). The accuracy of the MS/MS method for PCDD/F analysis as demonstrated by the analysis of the matrix spike is equivalent to HRMS method 8290 criteria (70% to 130% of actual value for tetra though congeners). In the case of the sediment extract analyzed, MS/MS was the only method that allowed quantitation of PeCDDs. The sample had mass interferants, tentatively identified as Hexa-PCBs that require mass resolution in excess of 49000 to be removed, and other unidentified materials evidenced by the observed source de-tuning.

The stability and reliability of mass spectrometry/mass spectrometry has been demonstrated by studies using PCDD/Fs as model compounds. The stability of signal generation is an instrumental quality, so one can conclude that the stability shown in this study would also carry over to the studies of other families of compounds (as long as the materials could undergo molecular fragmentation that gave rise to significant and stable product ions as in the case of $(M - COCl)^+$ with PCDD/Fs). This study demonstrated the enhanced selectivity of MS/MS over HRMS in the analysis of a particular compound (PeCDD). Additional examples of superior selectivity must be left to other researchers. The work has shown that, instrumentally, MS/MS is reliable and is especially useful in PCDD/F analysis of environmental samples that may be difficult to analyze completely by HRMS. The use of MS/MS for environmental analysis is suggested as a complimentary technique

to HRMS analysis of PCDDs and PCDFs, and not as a stand alone technique. The study covered the stability of the instrument for the analysis of tetra through octa congeners, although, were it to serve as a complimentary technique to HRMS, MS/MS would probably be most useful in the analysis of the lower mass congeners (tetra and penta), as that is where more mass interferants generally appear.

This study has shown that MS/MS is reliable and can be used on a regular basis. While the experiments were conducted on a hybrid instrument capable of high resolution mass spectrometry, the MS/MS experiments were conducted with the MS1 operating at a resolving power of 500-800. The significant implication is that, with MS/MS, regular part-per-trillion analysis of PCDD/Fs is possible without the necessity of a high resolution instrument.

While the study was able to demonstrate the long term reliability of our MS/MS instrumentation, it also high-lighted the difficulties that arise from the complexity of the instrument. The training required to perform MS/MS tuning and experimentation was more time consuming than that required for HRMS analyses. The additional analyzer and electronics used in the MS/MS experiments add to the complexity operation of the instrument and are certainly possible sources for error. All the experiments were carried out with the idea that, as long as the instrument was properly maintained and tuned, it should give reproducible data. The length of the study bore out this notion. Any problems (some subtle and intermittent) encountered

with relative response factors, isotope ratio errors, or drifting collision energy zero settings turned out invariably to be caused by either malfunctioning electronics or improper tuning.

Future work should include the repeated analysis of environmental samples on different instruments. Theoretically, analyses conducted on well maintained and tuned MS/MS instruments should yield similar results. Unfortunately, it was not possible in this study to perform analyses on other types of MS/MS instruments.

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